PHENOTYPING DROUGHT TOLERANCE IN CONVERTED *GOSSYPIUM* SSP. RACE STOCKS

A Thesis

by

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ABSTRACT

In cotton (*Gossypium hirsutum*), plant breeders seek means to reduce susceptibility to drought conditions through the incorporation of drought tolerance traits from exotic sources. A group of converted race stocks was phenotyped using high throughput techniques for traits that confer drought tolerance in order to characterize the variation among them and to determine the most advantageous growth stage for evaluating drought tolerance in terms of lint yield and fiber quality. Ten converted race stocks, two released cultivars, and two experimental elite lines were planted in three locations during 2015 and two locations in 2016 in a replicated field trial. Additionally, two high yielding strains were hybridized with four converted race stocks in a factorial mating design. Normalized Difference Vegetation Index, leaf surface temperature, stomatal conductance, and absolute chlorophyll content measurements were collected during the developmental stages of squaring, flowering, and boll development. Spearman's correlations were constructed and analyzed between lint yield, lint percent, micronaire, fiber length and strength and the drought tolerance traits for each growth stage while additive and dominance variation was calculated within the factorial mating design.

NDVI, leaf surface temperature and chlorophyll content showed a positive association with lint yield and lint percent during flowering while stomatal conductance showed association with lint yield and lint percent during boll development. Changes in fiber micronaire were closely related to differences with drought related effects during

boll development while fiber length and strength seemed to be affected by drought effects during flowering. Results of the factorial mating design study showed additive variation for leaf surface temperature, chlorophyll content and stomatal conductance while dominance variation exists for NDVI. The results of this study demonstrate potential within converted race stocks for traits that confer drought tolerance.

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NOMENCLATURE

CC	Corpus Christi
CRS	Converted Race Stock
CS-D	College Station – dryland
CS-I	College Station – irrigated
EUW	Effective Use of Water
GMA	Generation Means Analysis
HI	Harvest Index
HTP	High Throughput Phenotyping
NDVI	Normalized Difference Vegetation Index
WU	Water Use
WUE	Water Use Efficiency

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1. INTRODUCTION

In 2015, 1.295 million hectares of cotton (*Gossypium* spp.) were planted into non-irrigated fields in Texas, double that of the .607 million hectares that received irrigation (USDA/NASS, 2015). That same year, irrigated fields in Texas yielded an average lint yield of 1080 kg/ha compared to the non-irrigated yields of 503 kg/ha; nearly half the amount (USDA/NASS, 2015). During the drought of 2011 that affected the majority of US cotton production, Texas direct agriculture losses reached an estimated $5.2 billion with an additional $3.5 billion of indirect losses (Combs, 2012). The sensitivity of cotton and other crops to water deficient conditions presents an opportunity to identify drought tolerant traits from novel germplasm sources through modern phenotyping techniques.

Some past definitions of drought tolerance are based on measuring root and shoot growth rates, dry weights, leaf expansion (Ball et al, 1994), transpiration decline curves (Quisenberry et al., 1982), and boll counts (Pettigrew 2004) in an effort to indirectly measure the physiological effects of drought. In the emerging field of high throughput phenotyping, plant physiologists and agricultural engineers have collaborated to develop instruments that are capable of directly quantifying physiological characteristics in the field. High throughput phenotyping is a developing field, so few studies have been conducted. Application of these instruments represents an opportunity to discover variation in exotic germplasm that has been previously undetected. Since a plant's physiological response to water deficit conditions can change during development

(Blum, 1988), it is important to determine the associations between growth and various phenotypic traits.

Quisenberry (1981) evaluated a subset of the 1979 germplasm release by Jenkins, McCarty, Creech, and Parrot of converted race stocks and found variation among the race stocks for heat tolerance, root growth, dry matter accumulation, and water use efficiency under non-irrigated conditions. These race stocks have the potential to provide untapped variation for drought tolerance traits that, once identified, can be introgressed into already high yielding varieties. Before these traits can be transferred, it is important to determine how these quantitative traits are inherited so appropriate selection techniques can be utilized (Fehr, 1991).

Objectives

1. Evaluate converted race stocks for drought tolerant traits.
2. Determine critical growth stages for the most effective use of high throughput phenotyping of drought tolerance.
3. Develop estimates for additive and dominance gene action and narrow and broad sense heritability for drought tolerance, within the group of converted race stocks.

2. LITERATURE REVIEW

As the global population grows and climate change continues, many countries in arid regions with limited fresh water supplies will struggle to be self-sufficient in agricultural production (Falkenmark et al., 1998). It is increasingly important to develop methods in plant breeding for drought tolerance. However, drought tolerance is a highly complex physiological trait. The effects of drought can be confounded with weed and disease pressure, nutrient deficiencies, temperature extremes and expedited by soil structure, and agronomic management practices (Passioura, 2006). Under drought stress plants are affected on a holistic level, causing changes on multiple growth and developmental processes. Plant physiologists and breeders have categorized drought tolerance traits into concise breeding objectives that focus on resource use efficiency. The most efficient plant types can use available resources for the most gain in biomass (Quisenberry, 1981). In cotton, variability exists within converted race stocks that have the potential for providing needed variation for improving drought tolerance (Basal et al., 2005).

Causes of Drought Stress

The accumulation of CO_2 and other greenhouse gases in the atmosphere is leading to a rise in global temperatures (Shaftel, 2016). As global warming continues, it is projected that shifts will occur in soil moisture conditions. The Office of Technology Assessment (1993) projected an overall increase in precipitation of 7-15% with an

increase to global evapotranspiration of 5-10%. At low to mid-latitudes, evapotranspiration is predicted to exceed precipitation, leading to an increase in the frequency and severity of droughts in these areas (NDMC, 2016).

While the symptoms of drought stress are typically associated with insufficient water, they can also be born out of other stressors that affect crop production and indirectly lead to signs of water stress. Environmental stressors like salinity and low temperature can reduce conductance and limit plants ability to uptake water (Bohnert and Shevelena, 1998). Plant diseases that affect the root systems can inhibit root growth to the point where water deep in the soil profile cannot be absorbed by the root systems (Passioura, 2006). Through intense competition with weeds, plants can experience early onset of drought stress through the cumulative demand for resources (Patterson, 1995).

Soil texture and organic matter content are the two primary factors that affect a soil's capacity for holding water (Agvise, 2016). Soil texture is determined by the particle size distribution. Smaller particles like silt and clay have more surface area and can therefore hold more water compared to sand, which has larger particles. Soils with higher cumulative percentages of silt and clay thusly have a higher water holding capacity than primarily sandy soils. However, clay has a tendency to bind so tightly to water that it becomes unavailable to roots. Therefore, the ideal soil is typically a silt loam. Organic matter also affects the water holding capacity of a soil due to its affinity for water. As the amount of organic matter increases, independent of the soil texture, water holding capacity increases. During rainfall events, soils with lower water holding capacities become rapidly saturated. In these instances, the excess water is not attainable

by the root systems, leading to earlier signs of drought stress than in soils that are able to capture and hold higher quantities of water.

Cultural practices, such as cultivating, encourage evapotranspiration, depletion of organic matter, soil erosion and runoff. Through the adoption of conservation tillage, management of soil can be modified to reduce water loss. There are three main types of conservation tillage; no-till, ridge-till, and mulch-till as detailed by Janssen and Hill, (1994). Leaving the previous year's crop residue on the field reduces soil erosion by 60 to 90%, limits evapotranspiration at the soil surface, and increases soil's water holding capacity from the additional organic matter (MDA, 2016.) Through management practices like conservation tillage, it is possible to take a proactive approach in reducing a field's susceptibility to drought conditions.

Aquifers provide an important source of groundwater for irrigated agriculture, accounting for roughly 60% of the water used for crop irrigation in the United States (Scanlon et al., 2012). Due to extensive use by both agriculture and the general population, most aquifers in the United States are continuously pumped and rapidly becoming depleted. The Ogallala aquifer, that supplies most of the High Plains region of the United States, has dropped more than 30.5 meters in many areas between 1900 and 2008 (USGS, 2016). As groundwater availability is reduced without adequate rainfall for recharge, irrigated agriculture will increasingly give way to non-irrigated agriculture and associated to drought conditions.

In order to reduce groundwater use in irrigation systems, studies have been conducted to determine which systems are the most efficient at delivering water. Howell

(2003) defines irrigation efficiency in three parts: 1) system performance, 2) uniformity of water application, and 3) the crop response to irrigation. There are four main types of irrigation systems: flood (furrow and graded border) irrigation is directed throughout the field by raised beds and field borders; sprinkler (lateral, center pivot, traveling gun, and solid set) irrigation is delivered throughout the field with sprinklers either permanently installed within the field or upon machines that can traverse the field; drip irrigation typically consists of small tubing that directly applies small amounts of water to the root zone; and micro irrigation consists of small targeted sprinklers meant to irrigate only a specific area (ATS Irrigation, 2016). The crop being produced, soil structure, location, and cultural practices all influence the selection of an irrigation system for each situation (Onder et al, 2005).

Effects of Drought Stress

Ackerson, et al. (1977) concluded that the primary mechanism of action for drought stress is a drop in photosynthetic activity, attributed directly to insufficient water. The adaptiveness of photosynthesis to drought conditions depends on its pathway; C_3, C_4 or crassulacean acid metabolism (CAM). The C_3 photosynthetic pathway accounts for most of the known species, including rice (*Oryza sativa*), soybean (*Glycine max*), cotton (*Gossypium hirsutum*) and trees while C_4 plants (corn (*Zea mays*), crabgrass (*Digitaria sanguinalis*), sugarcane (*Saccharum officinarum*) and CAM plants like pineapples (*Ananas comosus*) are less common. These pathways are delineated by the location of Rubisco in relation to CO_2 fixation (Bear and Rintoul, 2016). In C_3 plants

the Calvin cycle and Rubisco are located near the site of CO_2 fixation in the mesophyll layer while in C_4 plants, they are located within the bundle sheath cells, separated from the CO_2. During periods of drought stress, the stomata will close and O_2 concentrations will increase within the cell. In C_3 plants, Rubisco fixes O_2 instead of CO_2 in a costly, inefficient process called photorespiration. C_4 plants do not experience photorespiration because Rubisco is separated from the mesophyll layer. The CAM plant's Calvin cycle is protected from photorespiration through time since stomata are closed during photosynthesis and Rubisco does not have access to O_2. Because of this difference, CAM and C_4 plants are considered to be less susceptible to drought stress than C_3 plants.

Stomatal closure is one of the first detectable signs that a plant is experiencing drought stress (Chaves et al., 2003) and can happen within minutes (Passioura, 1996). When stomata close in an effort to preserve water, oxygen and water vapor are no longer exchanged for CO_2, inhibiting the amount of assimilate that can be used toward plant growth or cotton fiber development. Species have different leaf water potential thresholds that trigger stomatal closure (Hsiao et al., 1979). Comparatively speaking, cotton has a greater ability to osmotically adjust and therefore maintain water potentials than other row crops (Oosterhuis and Wullschleger, 1988), enabling it to be economically productive in semi-arid climates. Through improved turgor maintenance under water stress, plants are still able to continue to develop, yet at slower rates than under optimum conditions (Sharp and Davies, 1979), enabling plants to potentially maintain root development and extract water from deeper within the soil profile (McMichael et al., 2011).

Plant growth is intrinsically tied to transpiration. The rate at which this occurs can depend on the climate, photosynthetic pathway, and leaf size (Rockström and Falkenmark, 2000). Ball et al. (1994) used leaf area and photosynthesis to monitor growth rates and detected a significant decrease during drought stress. In drought conditions, the physiological response of plants is determined in no small part by age of the plant (Blum, 1988). Many studies have been conducted on cotton's performance under water-deficit conditions in the field, greenhouse, and growth chamber to better understand the effects of drought on production.

Pace et al. (1999) found that cotton exposed to drought showed significant decreases in height, leaf area, nodes, and stem and leaf dry weights compared to their irrigated counterparts. Krieg and Sung (1986) saw a reduction in the total number of leaves on lateral branches in cotton. Pace et al (1999) and Ball et al (1994) determined that root elongation increased at the expense of root width yet plants still experienced an overall reduction in growth, development and distribution (Malik et al, 1979). McMichael and Lascano (2010) described how cotton roots are capable of "hydraulic lift" to bring water from lower in the soil profile to sustain roots in the drier sections of the soil to reduce the overall stress within the root system.

The root/shoot mass ratio is useful because it allows for a comparison of how different plants partition respective resources. Cook and El-Zik (1992) detected an inverse relationship between lint yield and the root/shoot ratio collected during first bloom. They inferred that cotton plants were partitioning resources to root growth as opposed to shoot and reproductive structures during water deficits. Conversely,

McMichael and Quisenberry (1991) and Pace et al. (1999) saw a decrease in the root/shoot ratio when drought stress was imposed near the end of development.

In a study of cotton conducted by Pettigrew (2004), drought stress reduced the total numbers of blooms, caused flowering to occur earlier and hastened cutout by as much as six days compared to the irrigated treatment. These results are similar to those found by Guinn and Mauney (1984), adding that flowering rates were unable to recover until three weeks following relief by irrigation. With a decreased amount of flowers and a slow rate of recovery, plants are unable to compensate for the loss. Lint yield is therefore highly associated with the number of flowers and bolls present and retained during drought stress (Grimes et al., 1969). Pettigrew (2004) confirmed this by finding that the principal factor responsible for yield loss during water deficits is the reduction in the number of bolls per plant. An intense nine day drought was imposed on cotton plants during the peak of flowering by Grimes et al., (1970) and was used to confirm that flowering was the most sensitive period of development for cotton in terms of its effect on lint production.

Fiber quality in cotton is determined by a combination of parameters designed to classify cotton fiber into grade standards. High volume instrument (HVI®) systems measure fiber samples for fiber length, length uniformity, strength, micronaire, color grade, trash, and leaf grade (Cotton Inc., 2016). Fiber length and micronaire are the quality properties most readily influenced by water deficits (Cotton Inc., 2016). Pettigrew (2004) found that fiber length is generally shortened in response to moisture deficits. Micronaire, a measurement of fineness and maturity, can be elevated in drought

years due to the excision of bolls due to stress (Hake et al., 1990). Each of the remaining bolls acts like a sink for the cellulose, leading to thicker fibers that take longer to mature.

Measuring Drought Stress

Passioura, (1996) defined drought tolerance "in terms of yield in relation to a limiting water supply" and offered Equation (1) to relate yield to terms of resource economics.

(1)

$$Yield = T \times WUE \times HI$$

where T is the amount of water transpired more commonly referred to as water use (WU); WUE is water-use efficiency, defined as "the ratio of above-ground dry matter to the amount of transpiration"; and HI is harvest index, defined as "the ratio of yield to above-ground dry matter". Water use, water use efficiency, and harvest index in this equation might appear to be independent but this is not the case (Condon and Richards, 1993). Estimates for transpiration efficiency are used in both WU and WUE, and biomass is also used in both WUE and HI calculations. Condon et al., (2004) demonstrated this relationship with the updated Equation (2):

(2)

$$Yield = ET \times (T/ET) \times W \times HI$$

where ET is evapotranspiration (the amount of water used in the crop), (T/ET) is the proportion of the total water transpired by the crop, W is the transpiration efficiency of biomass production, and HI is harvest index. Each of the four estimates can provide a potential target for genetic improvement (Condon et al., 2004). Blum (2009) took an alternative approach, however, concluding that selecting for the effective use of water (EUW) as opposed to WUE, is more appropriate because plants with high WUE are simply using less water and may have not undergone any increases in physiological performance (Blum, 2005). Blum (2009) defined EUW as the prioritization of biomass production during water deficits through improved soil water capture while maintaining functional stomatal transpiration. While WUE and EUW sound similar, functionally they have different effects on selection when applied in breeding. Selection for high WUE during periods of drought stress causes the population to shift toward accumulating traits that reduce total WU such as smaller leaf sizes and shorter developmental cycles (Blum, 2009).

High throughput phenotyping (HTP) provides an avenue for characterizing large numbers of genotypes in the field. Previously, attempts to characterize quantitative traits in large populations were only limited by our ability to phenotype the entire population within a reasonable amount of time (Araus and Cairns, 2014). HTP methods enable scientists using sensors (both handheld and mounted upon a vehicle) to collect non-destructive estimations for quantitative traits including yield potential and biotic and abiotic stress tolerance. While HTP allows for easy collection of multitudes of data,

synthesizing and analyzing these data proves more difficult and typically requires custom processing using software to interpret the data (White et al, 2012).

Remote sensing techniques can be used to gather data on a multitude of breeding objectives including yield estimates, abiotic (water stress, temperature extremes, nutrient deficiency, soil toxicity), and biotic stress (insect, animal, disease, nematodes) tolerance measurements, overall plant health, height, and approximate growth stage in a nondestructive manner (Araus and Cairns, 2014).

There are multiple types of cameras that can be used to collect images in the field. RGB/CIR cameras capture both visible and color infrared light, multispectral cameras can be used to monitor a limited number of spectral bands for use in calculating spectral indices, hyperspectral cameras are capable of capturing the entire electromagnetic spectrum between visible and near-infrared wavelengths, thermal cameras translate thermal radiation into a color scheme, and standard digital cameras can also be used for estimations of canopy density and senescence (Araus and Cairns, 2014). Laser imaging detection and ranging (Lidar) is a form of active remote sensing that constructs 3D images and enables measurement of plant height, cover, and canopy structure (Omasa et al., 2007). While the raw images are easily obtained using these cameras, post-processing can be time consuming due to the amount of work needed for accurate interpretation of the images (i.e. image alignment, calibrations, atmospheric corrections, mosaicking) (Araus and Cairns, 2014; Berni et al., 2009).

Spectral indices provide avenues for relating canopy reflectance to overall plant health in terms of biomass and leaf area index (Pinter et al, 1994). Normalized difference

vegetation index (NDVI) is calculated by subtracting the amount of visible red light (680 nm wavelength) reflected from the amount of near-infrared light (900 nm wavelength) reflected, then dividing that number by the sum of the two (Equation 3). It is considered a reliable indicator of biomass and overall plant health (Rouse et al., 1973; Gutierrez et al., 2012).

(3)

$$NDVI = (NIR - Red)/(NIR + Red)$$

Significant positive relationships with lint yield have been detected when it was compared to NDVI measurements taken throughout the growing season, suggesting the use of NDVI as a predictor for superior performing genotypes (Plant et al., 2000).

Producing estimations for stomatal conductance can provide valuable information to the photosynthetic health and efficiency of drought stressed plants. Handheld leaf porometers are able to estimate the rate of gas exchange and transpiration through the aperture of the stomata (Pask et al., 2012) and can be used to collect phenotypic information in the field. With each measurement taking roughly 30 seconds, this method is only moderately high throughput, but it allows scientists to gain non-destructive estimates for stomatal conductance. Quisenberry, et al. (1982) proposed that if differences in genotypes existed for stomatal behavior, then genotypes with higher rates of stomatal conductance would maintain photosynthetic productivity throughout longer periods of the day and therefore demonstrate improved performance. Caution does need to be taken when collecting stomatal conductance measurements because of

the sensitivity of the stomata to touch, light, heat, CO_2 concentration, and leaf water status (Chaves et al., 2003).

Another form of estimating stomatal conductance relies on leaf surface temperature's inverse relationship with stomatal conductance (Lu et al., 1994). However, studies have been conducted that focused on canopy temperature's efficacy as an indicator for drought tolerance (Singh and Kanemasu, 1983; Jackson et al., 1981). Ehrler (1973) suggested that irrigation schedules could be constructed around the linear relationship formed from the difference of air and leaf temperature during water deficits. Congruent with the conclusions of Hatfield et al. (1987) that relative canopy temperature is influenced by the amount of soil water available. Varieties with warmer leaf temperatures earlier in the growing season experience less transpirative cooling due to lower rates of water use. However, these varieties, hypothetically, should maintain available soil water later into the growing season and have cooler leaf temperatures compared to varieties with greater water use (Hatfield et al., 1987)

In order to synchronously collect multiple types of data, sensors and cameras have to be mounted and programmed to function on high throughput phenotyping platforms (HTPP), which can range from ground based to aerial systems (Araus and Cairns, 2014). The ground based platforms typically consist of modified field vehicles that have mounted sensors and include a GPS system to spatially link the data points. The ground based approach enables plot-level precision and provides the most easily processed data, but also requires more time in the field; exposing the data to error caused by environmental variation over time. Aerial platforms can resolve this issue by

capturing an entire field within minutes but most platforms are only able to carry a small payload and can be more expensive to purchase and maintain. (White et al, 2015).

While there are still challenges to high throughput phenotyping, recent studies have shown that substantial progress is being made to bring these techniques into breeding programs. Andrade-Sanchez et al. (2014) described a high throughput phenotyping platform that collects simultaneous measurements of plant height, temperature and reflectance, and used the data to make comparisons between the genotypes and calculate heritability estimates. Carmo-Silva et al. (2012) used a high clearance tractor mounted with radiometric infrared thermometers to collect temperature data for use in determining stomatal conductance. Pauli et al. (2016) used the phenotyping platform described by Andrade-Sanchez et al. (2014) to map quantitative trait loci (QTL) for drought tolerance traits, and detected changes in QTL expression based on developmental stages. With improving technology, it is becoming more feasible to incorporate HTP techniques to expedite selection for favorable traits in plant breeding programs.

Root measurements are more difficult to collect than leaf measurements simply by nature of location. In oak *(Quercus spp.)*, ground-penetrating radar (GPR) has been used to render 3D images of root systems to estimate biomass without needing to harvest the root manually (Hruska et al., 1999); but in cotton, studies were conducted using mini-rhizotrons (McMichael, 1990; Keino et al., 1995) that allow for the nondestructive viewing of root systems (Johnson et al, 2001) in the field.

It is a combined goal of plant breeders and plant physiologists to develop screening methods that can be performed at the seedling growth stage to expedite selection. Longenberger et al. (2006) described a screening method developed for growth chambers that subjected cotton seedlings to multiple controlled drought cycles to detect differences for drought tolerance on an individual plant basis. Basal et al. (2005) planted cotton seedlings into greenhouse pots to quantify root response and excised leaf water loss to differentiate germplasm response to water deficits. Due to the complexity of drought stress, an ideal seedling screening method is difficult to isolate and work needs to be conducted to develop more efficient systems.

Epicuticular wax load has been shown to have a strong linear relationship (r = .72 in irrigated and r = .94 in non-irrigated conditions) with water use efficiency in sorghum (Premachandra et al., 1994). While not a significant source of water loss in typical conditions, transpiration through the cuticle of a leaf can amount to more water loss than through partially or closed stomata in water limited conditions (Nobel, 1991). In peas (*Pisum sativa, L.)*, significant negative relationships were discovered between epicuticular wax load and leaf temperature (Sanchez et al., 2001). Bondada et al. (1996) found that wax concentration in cotton leaves, bracts, and bolls increased when growing in water deficit conditions.

Leaf chlorophyll content can provide an avenue of selection for higher yielding varieties (Singh, 2001) yet its exact relationship with drought tolerance requires further study (Karademir et al., 2009). As a major component of chloroplasts, the amount of chlorophyll has a positive relationship with the rate of photosynthesis (Guo and Li,

1996). Differences exist between genotypes of barley (*Hordeum vulgare* L.) for the adaptability of photosynthesis components during drought stress (Rong-hua et al, 2006). Leaf chlorophyll concentration measurements can be collected rapidly in any location using handheld meters (Parry et al., 2014).

Breeding for Drought Tolerance

Water deficits typically take two forms; occurring for either a short time or developing over a long period of time. Plants' methods for coping involve a mixture of avoidance and tolerance mechanisms that can vary among genotypes (Chaves, 2002). Typically, avoidance includes strategies that allow plants to escape physiological deficits through shortened developmental cycles or by optimizing plant growth to match resource availability, while tolerance mechanisms include methods of enduring deficits by stomatal closure or leaf shedding and curling to reduce light absorbance (Chaves, 2003). Natural selection for drought tolerant traits would yield plants with improved adaptability and survivability; however, human selection favors improved yield potential (Cattivelli et al., 2008).

When designing selection methods for drought tolerance, it is always an important consideration to be able to phenotype germplasm in the field as efficiently as possible to detect differences and select the superior progeny (Araus and Cairns, 2014). This goal is even more complicated by the knowledge that drought tolerance is often in tandem with heat, light, insect, and disease stressors, keeping quick and efficient screening methods from being developed (Longenberger et al., 2006). The complexity

of breeding for drought tolerance hinders the amount of progress that can be achieved in improving crop performance in drought areas (Cattivelli et al., 2008).

Many plant breeders believe that superior varieties in irrigated conditions will also be superior in non-irrigated conditions. Araus et al. (2002) found that selection for high yield potential in cereals led to a concomitant improvement in yield in drought conditions. Similar findings have also been reported in wheat (*Triticum sativa* L.), barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) (Slafer and Whitechurch, 2010; Tambussi et al., 2005; Trethowan et al., 2002). We could infer from these results that traits that result in enhanced production efficiency in well-watered conditions also confer efficiency when water is limited. Thusly, superior genotypes in irrigated fields will likely be the superior genotypes in non-irrigated fields.

The first step in designing a breeding program for drought tolerance is to determine the selection parameters upon which a plant breeder will focus their efforts. Most plant breeding programs will compare yields in both irrigated and non-irrigated conditions and incorporate traits that explain differences in yield in terms of WU, WUE, and HI. Some examples of traits often used in studies to phenotype drought tolerance include NDVI (Gutierrez et al., 2012; Karneli et al., 2010), canopy temperature (Singh and Kanemasu, 1983; Hatfield et al., 1987), stomatal conductance (Carmo-Silva et al., 2012), chlorophyll content (Said, 2014; Karademir et al., 2009), or a combination herein (Pauli et al., 2016; Andrade-Sanchez et al., 2014).

To facilitate selection in a breeding program, it is helpful to understand how traits are inherited (Fehr, 1991). Mating designs (design I, design II, and diallel) in plant

breeding studies can be used to develop estimates for additive and dominance variation and heritability and of targeted traits based on how traits segregate in different generations (Bernardo, 2010). A generation means analysis (GMA) allows it to be taken a step further and also determine epistatic interactions between the additive and dominance effects; namely the additive x additive, additive x dominance, and the dominance x dominance effects. Said (2014) used a GMA in wheat to determine the potential for genetically improving drought tolerance traits and showed epistatic interactions were significant for every trait analyzed. In cotton, GMA have been used to evaluate the germplasm potential of converted race stocks (CRS) for yield components (Ragsdale and Smith, 2007) and fiber quality properties (Hwa, 2013).

Another approach to better understand drought tolerance involves the use of quantitative trait loci (QTL) mapping. Saranga et al. (2001) hybridized *Gossypium hirsutum* and *Gossypium barbadense* and detected a total of 161 QTLs in the progeny, and 102 of these showed no differential expression between their irrigated and non-irrigated trials. Of the remaining 59 QTLs, 33 influenced performance under water limited conditions, 13 determined performance in irrigated conditions, and the remaining 13 related to the ratio of performance between locations. Pauli et al. (2016) utilized HTP to take repeated measurements of a QTL mapping population of cotton and discovered differential expression of QTLs depending on the growth stage. With the presence of QTLs that can be related to drought tolerance in cotton, it is becoming easier to understand the underlying mechanisms of drought tolerance and to formulate selection practices to best capture drought tolerance within germplasm.

Use of Exotic Cotton Germplasm

Genotypic variability is limited among improved germplasm for traits that can improve drought tolerance and water use efficiency within advanced cotton germplasm (Quisenberry, 1981; McCarty et al., 2007). Continual reselection out of elite germplasm creates a genetic bottleneck, which limits genetic gain while increasing genetic vulnerability (McCarty et al., 2007). It is necessary to look at alternative sources of drought tolerant germplasm to increase diversity available to plant breeders.

There are four primary cultivated species within the *Gossypium* genus; *G. arboreum, G. barbadense, G. herbaceum,* and *G. hirsutum* (Smith and Cothren, 1999). *G. arboreum* and *G. herbaceum* are typically referred to as the 'Old World' species and are diploids. Prior to domestication, a chance outcross event between the two diploid species gave rise to the allotetraploid 'New World' species, *G. barbadense* and *G. hirsutum* that account for more than 90% of the total cotton acreage worldwide (Smith and Cothren, 1999).

Within *Gossypium hirsutum* there are seven land races; 'palmeri', 'morilli', 'richmondi', 'yucatenanse', 'punctatum', 'marie-galante', and 'latifolium' (Khandi et al., 2009), distinguished by growth habit (Smith and Cothren, 1999). Race 'palmeri' is often distinguished by its laciniate leaf shape, prolific flowering and small bolls. Race 'morilli' branches heavily and appears more round in growth habit. Race 'richmondi' is often a large, heavily branched shrub with medium size bolls. Race 'yucatenanse' is the most primitive of the landraces and is a small subshrub. Race 'punctatum' is narrow-stemmed and capable of large numbers of small to medium bolls with short fibers. Race

‘marie-galante’ is the most tree-like of the land races with a dominant central stem. Lastly, race ‘latifolium’ is a subshrub with medium to large bolls and is the least photoperiodic of the land races. These land races were formed through domestication, starting as wild plants possessing attractive traits that were cultivated, incorporating useful alleles into the gene pool (Tanksley and McCouch, 1997) and have the potential to provide new sources of variation (Iqbal et al., 2001).

Before these photoperiodic races can be readily used for trait introgression in a cotton breeding program, they must be converted to day neutrality. Once converted, they are referred to as converted race stock (CRS) (McCarty and Jenkins, 1993). The conversion process requires an initial cross to a photoperiod insensitive cultivar that flowers appropriately in the target location. Repeated backcrosses to the wild parent are necessary in order to recover as much of the original land race genotype while always selecting for flowering in the target location. While CRSs may possess traits for drought tolerance, variation in exotic germplasm typically comes hand in hand with unfavorable linkage groups for desirable agronomic traits. Drawbacks to using CRSs include an inconsistent loss of the original exotic parent’s variation during the conversion process and the original exotic lines were neither homozygous nor homogenous (Ragsdale and Smith, 2007). This genetic inconsistency within and between the CRS lines can cause difficulties during incorporation into breeding programs.

Regardless, there are successful examples of incorporating exotic germplasm into breeding programs. The Germplasm Enhancement of Maize (GEM) project is meant to incorporate novel traits in exotic germplasm in an effort to increase variation and

therefore improve U.S. hybrid corn performance (Pollak and Salhuana, 1998). Singh et al (2001) evaluated heterosis from crosses between established corn inbreds and GEM lines, determining that while not all of the hybrids performed acceptably, a few showed potential if used in the right inbred combination. The Sorghum Conversion Program is another example of successful incorporation of exotic germplasm, converting 840 exotic lines to be used in sorghum improvement programs (Klein et al., 2008).

In cotton, the *G. hirsutum* land races have been evaluated for potential uses. McMichael et al. (1984) investigated leaf initiation rates, leaf growth, and dry weights and demonstrated that variability exists between the exotic strains. Quisenberry et al., (1981) showed that significant variation exists for above ground dry matter accumulation, heat tolerance, root and shoot growth, and water use efficiency during periods of drought stress. McMichael and Quisenberry (1991) performed an analysis of cotton root systems in the greenhouse and found significant variability for root size along with genotypic differences in the root-shoot ratio. Cotton vascular bundles typically have four xylem bundles, but McMichael et al. (1985) discovered that one strain of 'punctatum', T25, possessed five bundles. When investigated further, McMichael et al. (1987) concluded the additional vascular bundle positively influenced lateral root development. These studies demonstrate that useful variation exists within the population of CRSs which can be used to improve drought tolerance in modern elite germplasm.

3. MATERIALS AND METHODS

In order to assess drought tolerance in a group of CRS lines, this study was divided into three experiments; high throughput phenotyping, generation mean analysis, and factorial. The high throughput phenotyping study evaluated the CRS lines for normalized difference vegetative index (NDVI), leaf temperature, chlorophyll content, and stomatal conductance traits when exposed to drought conditions. The generation mean analysis and factorial determined the heritability of these traits and the amount of variation that can be attributed to additive or dominant gene action.

High Throughput Phenotyping

Fourteen genotypes were evaluated for drought tolerance. Ten converted race stocks were selected to reflect diversity of race designations within *Gossypium hirsutum* from the USDA-ARS National Cotton Germplasm Collection located at College Station, TX. Each race stock is the product of a cross between an exotic parent and either 'Deltapine 16' or 'Lubbock Dwarf' with repeated backcrosses to the exotic parent always selecting progeny that flower in the southern United States (Jenkins et al, 1979, McCarty and Jenkins, 1993). Table 1 contains a list of converted race stocks including the race designation of the original exotic parent to show the diversity of the converted race stocks used within the study. The remaining four entries consist of 'Tamcot 73' (Smith et al., 2011), 'DP 491' (PVP 200100159, PI 618609) and two elite germplasm

lines, ‘10 X-64’ and ‘10 X-78’, developed at the Cotton Improvement Lab at College Station, TX.

Table 1. Converted race stocks used in the HTP study, 2015 and 2016.

Registration Number	NSL* Number	Line Name	Race Designation
GP 67	109637	JPM-782-26-2	punctatum
GP 76	109653	JPM-786-295-2	morilli
GP 79	109644	JPM-784-336-2	palmeri
GP 116	109642	JPM-782-1045-2	punctatum
GP 122	109608	JPM-781-66-1	latifolium
GP 130	109623	JPM-781-109-1	latifolium
GP 137	109635	JPM-782-25-1	punctatum
GP 138	109640	JPM-782-488-1	punctatum
GP 140	109646	JPM-785-461-1	richmondi
GP 561	561999 (PI #)	M-9044-0164	hirsutum

*NSL – National Seed Laboratory

Locations

Entries were planted in a randomized complete block design (RCBD) with four replications at the Texas A&M AgriLife Research Stations at College Station and Corpus Christi, TX, in 2015 and 2016. Two row plots were planted at all locations in 2015 but only in College Station in 2016. One row plots were used in Corpus Christi in 2016. Plots in Corpus Christi were 11 m in length with 1 m row spacing while College Station consisted of 13 m length rows with 1 m row spacing in each year. Standard management practices for each location were utilized. In both years, irrigated and non-irrigated tests were planted in College Station where the soil type is a Weswood silt

loam, characterized as a fine-silty, mixed, superactive thermic Udifluventic Haplustepts. Corpus Christi was non-irrigated for 2015 and 2016 and has a Victoria Clay defined as a fine, smectitic, hyperthermic Sodic Haplusterts (Soil Survey, 2016).

Sensors

Four sensors were used to characterize drought tolerance in this study. A Trimble Handheld GreenSeeker®, an Apogee Infrared Radiometer (Model MI-220), a Decagon Devices Steady State Diffusion Porometer more commonly called a Leaf Porometer®, and an Apogee Chlorophyll Content Meter (Model CCM-200).

The GreenSeeker emits red and near-infrared light when triggered, measures reflectance in the wavelengths emitted, and calculates NDVI (Trimble, 2016). The GreenSeeker was used both in the morning between 8:00 and 9:30 and the afternoon between 13:00 and 14:00. It would take approximately 30 minutes to complete each round of data collection with this device. Averages for each row were obtained automatically through continuous measurements taken by the sensor when the trigger is held. Optimum NDVI measurements are collected when the sensor is held in front of the user, 0.6 to 1.2 meters above the row (Trimble, 2016).

Leaf surface temperature measurements were obtained with the radiometer. Infrared radiometers (IRRs) measure surface temperature by producing an electrical signal based on thermal energy captured within their field of view (Apogee, 2016b). In the case of the Apogee MI-220, thermal energy within the 38 degree field of view is averaged to produce an estimate (Apogee, 2016b). Measurements were collected from

each plot by directing the sensor to the uppermost fully-mature leaf on three randomly selected plants and averaging them together to produce a plot temperature. Cloud cover can cause discrepancies in the data by decreasing the amount of solar radiation that reaches the field and therefore impacting leaf surface temperature. Therefore, in the case of cloud cover, data collection was postponed until the plots were once again in direct sunlight for several minutes. Temperature data was collected in both the morning and the afternoon, starting as close to 9:00 and 14:00 as possible, taking roughly 40 minutes to complete.

The Chlorophyll Content Meter provides an estimate of chlorophyll present within the 71 mm^2 measurement area in the form of the chlorophyll concentration index (CCI) (Apogee, 2016a). When CCI is plotted against a graph of absolute chlorophyll, a non-linear relationship exists. In order to ensure that data collected fits a linear model, CCI can be converted to absolute chlorophyll using the formula in Equation 2 to linearize the data (Parry et al., 2014).

(4)

$$Absolute\ Chlorophyll\ (\mu mol/m^2) = -84.3 + 98.6\ (CCI)^{.505}$$

Measurements were collected from the uppermost fully mature leaf of three different plants to produce an average for each plot. Collection would typically begin between 12:30 and 13:00 in the afternoon and would take approximately 45 minutes to complete.

The leaf porometer is used to measure stomatal conductance in $mmol/m^2s$. Once the clip is placed on the leaf, two relative humidity sensors work in unison to estimate

the rate of CO_2 moving through the stomata and out into the environment in 30 seconds (Decagon, 2013). Measurements were taken with the leaf porometer during the time of 10:00 to 14:00, when the plant was near its photosynthetic peak. Data collection with this device was typically completed within 3 hours.

Analysis

The statistical analysis was completed using JMP® Pro 12 (SAS Institute Inc., Cary, NC, 1989-2007). For each of the traits investigated, the model: Trait = Genotype + Growth Stage + Location + Rep (Location) + Location*Genotype + Year + Error was used. Fisher's LSD was performed to determine whether there were significant differences among genotypes. As differences were determined, Spearman correlations were constructed among measured drought tolerance traits at each growth stage (squaring, flowering, and boll development) and lint yield, lint percent, and fiber quality traits such as micronaire, length, and strength.

Generation Mean Analysis and Factorial

Location

In 2016, entries were planted at the Texas A&M AgriLife Research Station at College Station, TX, in an RCBD with three replications in a non-irrigated field for both the Generation Mean Analysis (GMA) and factorial studies. Each replication consisted of single row plots of 14.33 m length and 1 m row spacing. Typical recommended field management practices were implemented.

Sensors

It is important to note that readings from sensors can be affected substantially by the responses of plants to diurnal variation. Therefore it is critical to standardize the daily data collection times to minimize experimental errors. The GreenSeeker was used to characterize NDVI measurements within each family. The GreenSeeker was used both in the morning and the afternoon. Start times fell between 8:00 and 9:30 and 13:00 and 14:00 in the afternoon. Data collection with this device for both studies took an hour each to complete.

The IRR was used to measure leaf surface temperature between the generations in each family. Temperature data was collected in both the morning and the afternoon, starting close to 9:00 and 14:00 taking up to 2 hours to complete both GMA and factorial studies.

The Chlorophyll Content Meter was used to collect chlorophyll measurements in terms of chlorophyll concentration index (CCI) (Apogee, 2016a). Measurements were converted to absolute chlorophyll using Equation 2. Data collection with this device can be completed in 2 hours for both studies.

The Leaf Porometer was used to estimate stomatal conductance (Decagon, 2013). Due to the size of this study and the importance of collecting data during the peak of photosynthesis, measurements in each of the three replications were collected from 10:30 to 14:00 on three consecutive days, one replication per day.

Generation Mean Analysis

Crosses were made in 2014 between four of the Cotton Improvement Lab's (CIL) elite germplasm lines and GP 137 to form four F_1 populations (Table 2). During the 2015 field season, these F_1 populations were regenerated and back-crossed to each parent to generate BC_1F_1 populations for each parent in each family. F_1 seed was planted in the greenhouse during the 2015-16 winter to create the F_2 generation.

Table 2. Entries used in generation mean analysis in 2016.

Family	Name	Pedigree	Role
Parents	10X-63	CIL Elite Germplasm	P_1
	10X-64	CIL Elite Germplasm	P_1
	07X-26-3	CIL Elite Germplasm	P_1
	10X-78	CIL Elite Germplasm	P_1
	GP 137	JPM-782-25-1	P_2
1	15105	10X-63/GP 137	F_1
	16WGH-05	15105	F_2
	15101	10X-63/GP 137//10X-63	BC_1P_1
	15121	10X-63/GP 137//GP 137	BC_1P_2
2	15107	10X-64/GP 137	F_1
	16WGH-06	15107	F_2
	15103	10X-64/GP 137//10X-64	BC_1P_1
	15123	10X-64/GP 137//GP 137	BC_1P_2
3	15108	07X-26-3/GP 137	F_1
	16WGH-07	15108	F_2
	15104	07X-26-3/GP 137//07X-26-3	BC_1P_1
	15124	07X-26-3/GP 137//GP 137	BC_1P_2
4	15119	10X-78/GP 137	F_1
	16WGH-18	15119	F_2
	15102	10X-78/GP 137//10X-78	BC_1P_1
	15122	10X-78/GP 137//GP 137	BC_1P_2

Analysis

All statistical analyses were completed with JMP Pro 12 (SAS Institute Inc., Cary, NC, 1989-2007). Generation means and variances were separated using Fisher's LSD for each trait studied at each growth stage. Families with significant differences among generations were used for the generation mean analysis (GMA). 'ABCD' scaling tests were used to determine whether the three parameter or the six parameter model is most appropriate for each family (Mather, 1982). A and B test for additive x dominance epistatic interactions. C weighs the dominance x dominance interactions, while D is additive x additive (Mather, 1982).

(5)

$$A = 2BCP_1 - P_1 - F_1$$

$$B = 2BCP_2 - P_2 - F_1$$

$$C = 4F_2 - 2F_1 - P_1 - P_2$$

$$D = 2F_2 - BCP_1 - BCP_2$$

Once values were obtained for each test, the variance for each test was calculated. The adequacy of the three parameter model is established if zero is within the values for A, B, C, and D plus and minus the standard error. Standard errors were calculated by taking the square root of each test as calculated in Equation 4.

(6)

$$V(A) = 4(V(BCP_1)) - V(P_1) - V(F_1)$$

$$V(B) = 4(V(BCP_2)) - V(P_2) - V(F_1)$$

$V(C) = 16(V(F_2)) – 4(V(F_1) – V(P_1) – V(P_2)$

$V(D) = 2V(F_2) – V(BCP_1) – V(BCP_2)$

Depending on the results of the scaling test, generation means were used to calculate the mid parent value (m), additive effects (a), dominance effects (d), additive x additive interaction (aa), additive x dominance (ad), and dominance x dominance (dd) for each family as shown in Equation 5. If the scaling tests proved that the three parameter model was adequate, only the mid-parent value, additive effect, and dominance effect were calculated (Gamble,1962).

(7)

Mid-parent Value (m) $= \mu F_2$

Additive Effects (a) $= \mu BCP_1 - \mu BCP_2$

Dominance Effects (d) $= -(\mu P_1/2) – (\mu P_2/2) + \mu F_1 – (4\mu F_2) + [2(\mu BCP_1 + \mu BCP_2)]$

Additive x Additive Effects (aa) $= -(4\mu F_1) + [2(\mu BCP_1 + \mu BCP_2)]$

Additive x Dominance Effects (ad) $= -(\mu P_1/2) – (\mu P_2/2) + \mu BCP_1 + \mu BCP_2$

Dominance x Dominance Effects (dd) $= \mu P_1 + \mu P_2 + (2\mu F_1) + (4\mu F_2) – [4(\mu BCP_1 + \mu BCP_2)]$

Factorial

Hybridizations were made in 2015 to generate F_1 progeny in a factorial mating design as described by Fehr (1991). Two lines, 10X-78 and ‘Tamcot 73’, used solely as females, were selected based upon high-yield potential, while GP 76, GP 122, GP 137,

and GP 140 were used as males to represent the CRS. Eight F_1 progeny were generated from this mating design (Table 3)

Table 3. Entries used in factorial analysis in 2016.

Name	Pedigree	Role
10X-78	CIL* Elite Germplasm	Female
Tamcot 73	TAAR**	Female
GP 76	JPM-786-295-2	Male
GP 122	JPM-781-66-1	Male
GP 137	JPM-782-25-1	Male
GP 140	JPM-785-461-1	Male
15113	Tamcot 73/GP 76	F_1
15114	Tamcot 73/GP 122	F_1
15115	Tamcot 73/GP 137	F_1
15116	Tamcot 73/GP 140	F_1
15117	10X-78/GP 76	F_1
15118	10X-78/GP 122	F_1
15119	10X-78/GP 137	F_1
15120	10X-78/GP 140	F_1

*CIL – Cotton Improvement Lab
**TAAR – Texas A&M AgriLife Research

Analysis

Data collected was analyzed using JMP Pro 12 (SAS Institute Inc., Cary, NC, 1989-2007). Estimates for additive and dominance variation for each trait observed were calculated following the procedure outline by Bernardo in 2010. First, mean square values were determined by utilizing the model; *trait = Rep + Males + Females + Males x Females + Error*.

Mean square values were used to derive V_m (variation due to the males), V_f (variation due to female), and V_{mf} (Variation due to the interaction of males and females). V_a and V_d were then calculated to estimate overall variation due to additive and dominance gene action for each trait analyzed as calculated in Equation 6 (Bernardo, 2010):

(8)

$$V_m = (1/r \times f) \times (MS_m - MS_{mf})$$

$$V_f = (1/r \times m) \times (MS_f - MS_{mf})$$

$$V_{mf} = (1/r) \times (MS_{mf} - MS_e)$$

$$V_{a(males)} = 4 * V_m$$

$$V_{a(females)} = 4 * V_f$$

$$V_d = 4 * V_{mf}$$

where r = reps, m = number of males, and f = number of females.

4. RESULTS, DISCUSSION AND CONCLUSIONS

During this study, College Station and Corpus Christi received above average, monthly rainfall in 2015 and 2016 (Table 4) as reported by US Climate Data (2016). In April and May of both years, Corpus Christi and College Station received above average rainfall. July precipitation, in both years at both locations, was well below the long-term average. Late June and July coincided with cotton flowering stages in both years. Flowering was shown by Grimes et al. (1970) and Pettigrew (2004) to be the most sensitive growth stage for drought stress that can affect lint yield. Nearing harvest in September of both years and locations, precipitation was below the long-term average for each location. Although there are periods where rainfall was below the average, drought stress was not apparent at either location in 2015. Plants were not appreciably stunted in growth or wilted. In 2016, however, symptoms of drought stress were visible at both locations at the end of July and August during data collection.

High Throughput Phenotyping

Due to poor stand establishment, weed pressure, and herbicide damage, the 2016 irrigated trial was dropped from the study. Analysis of model effects for lint yield and fiber quality traits (Table 5) show that genotype had a significant effect on the model for lint yield, lint percent and all fiber traits. Location was significant for lint yield and all fiber traits. Year and genotype x location effects were not significant for any of the

Table 4. Average monthly maximum and minimum temperatures and rainfall for College Station and Corpus Christi, TX in 2015 and 2016.

College Station, TX	Month					
	April	**May**	**June**	**July**	**August**	**September**
			2015			
High Temp (Cº)	26.33	28.66	32.33	34.83	36.17	33.39
Low Temp (Cº)	16.55	19.66	22.78	24.17	23.72	21.72
Rainfall (mm)	121.92	247.14	132.33	7.87	34.54	44.20
			2016			
High Temp (Cº)	26.22	28.11	33.11	35.89	32.44	32.78
Low Temp (Cº)	15.22	29.61	23.17	24.83	22.44	22.78
Rainfall (mm)	137.67	328.42	65.53	6.10	226.57	48.51
Avg. Rainfall (mm)†	71.12	99.06	120.90	60.20	70.87	85.34
Corpus Christi, TX			**2015**			
High Temp (Cº)	26.94	29.56	32.28	34.78	35.17	33.00
Low Temp (Cº)	19.83	22.78	24.11	24.72	24.28	23.17
Rainfall (mm)	161.54	363.73	41.40	30.23	74.42	62.48
			2016			
High Temp (Cº)	28.39	30.56	33.06	34.50	34.72	34.44
Low Temp (Cº)	19.06	22.39	24.06	25.94	25.11	24.44
Rainfall (mm)	86.36	152.15	74.93	0.00	104.65	77.47
Avg. Rainfall (mm)†	46.74	78.00	85.34	70.90	74.17	126.49

† Average rainfall over last 30 years

Table 5. Mean square errors for agronomic and fiber quality properties of converted race stocks at College Station and Corpus Christi, TX in 2015 and 2016.

Model Effects †	Lint Yield d.f.	Lint Yield	d.f.	Lint Percent	Micronaire	Length	Strength
	-	kg/ha	-	%	units	mm	kN/m kg
Genotype	13	2,277,047**	13	.0071**	.245**	12.85**	394.2**
Location	2	6,398,458**	2	.0003	2.396**	16.48**	621.0**
Rep [L]	9	1,226,695**	5	.0015**	.079	1.12	46.8
Year	1	130,925	1	.0001	.007	.84	1.5
G x E	26	212,655	26	.0001	.050	.65	25.7
G x Y	13	835,273**	13	.0002	.040	.40	19.0
Error	215	233,398	79	.0001	.067	.83	28.5
C.V. (%)	-	36.7	-	3.5	3.4	3.2	5.4

*, ** denotes significance at the .05, .01 level respectively.

† C.V., coefficient of variation; Rep, replication; L, location; G x E, genotype x location; G x Y, genotype x year; d. f., degrees of freedom

measured variables. Genotype x year was significant for lint yield and was likely caused by differences in seed quality between years. The seed used in 2015 had lower germination rates compared to seed used in 2016.

The four elite lines and cultivars were among the highest yielding entries (Table 6). GP 561 was the closest among the CRS lines in terms of lint yield to the four elite lines. All CRS lines had lower lint percent than the four elite lines, which partially explains the CRS lines' limited utility as breeding stock. Lint percent is an important yield component. Fiber quality varied among CRS lines, but most tended to be of lower quality than the four elite germplasm lines, especially for fiber strength and length. GP 116 had the shortest fiber length and ranked among the lowest for micronaire and strength.

Upon comparison of location effects upon the study, it was apparent that the dryland trials at College Station resulted in the highest lint yields (Table 7). This was partially the result of the improved seed quality of 2016 versus 2015. Since the irrigated trial at College Station in 2016 was not included in the averages, only yield from the lower seed quality of 2015 is impacting the results. The dryland trials at Corpus Christi appeared to have a negative effect upon fiber length and strength compared to those grown at College Station. The irrigated trial at College Station produced fibers with the highest micronaire, which may be the result of thin stands (Bednarz et al., 2004) in 2015. Location was not a factor that affected lint percent among the lines in this study.

Table 6. Fisher's LSD for agronomic and fiber quality properties by genotypes across locations in 2015 and 2016. Mean values followed by different letters are significantly different at the .05 level.

Genotypes	**Lint Yield**	**Lint Percent**	**Micronaire**	**Length**	**Strength**
	kg/ha	%	units	mm	kN/m kg
Tamcot 73	2010a	37.0b	4.6bc	29.6a	348.9a
10X-64	1956a	38.8a	4.6bc	29.5a	337.1ab
DP 491	1885a	39.1a	4.6bc	29.4a	327.3bcd
10X-78	1678ab	38.2ab	4.7bc	28.7ab	331.2bc
GP 561	1353bc	35.5c	4.7ab	27.5cde	294.0g
GP 67	1284cd	33.9d	4.8ab	27.6cde	305.8efg
GP 79	1176cde	32.1fg	4.6bc	27.0de	301.8fg
GP 76	1173cde	32.5ef	4.6bc	28.2bc	307.8efg
GP 130	1166cde	33.6de	5.0a	26.8e	292.0gh
GP 116	1113cde	31.0gh	4.1c	25.2f	274.4h
GP 140	1004cdef	34.8cd	4.8ab	27.8cd	315.6cdef
GP 137	984def	31.0gh	4.8ab	27.1de	311.6def
GP 138	882ef	33.8d	4.7abc	27.7cde	301.8fg
GP 122	730f	30.5h	4.9ab	27.3cde	322.4bcde
Mean	1314	34.4	4.7	27.81	312.6

Table 7. Lint yield, lint percent and fiber qualities of converted race stocks at College Station and Corpus Christi in 2015 and 2016. Mean values followed by different letters are significantly different at the .05 level.

Locations †	Lint Yield	Lint Percent	Micronaire	Length	Strength
	kg/ha	%	units	mm	kN/m kg
CS-D	1559a	34.3a	4.8b	28.2a	321.4a
CS-I	1289b	35.0a	5.0a	28.3a	318.5a
CC	1081b	34.3a	4.4c	27.2b	299.9b
Mean	1314	34.4	4.7	27.8	312.6

† CS-D, College Station – dryland; CS-I, College Station – irrigated; CC, Corpus Christi

In terms of traits measured by sensors, NDVI readings taken both in the morning and afternoon were affected by genotypes, locations, years, growth stages, and genotype X year interactions (Table 8). These results are not surprising since NDVI is a combination of both leaf area and photosynthetic capacity within a given area, which can be affected by the environment, plant age, and genetic influence of a particular plant. Leaf temperature was different in the morning per location (Table 8). There are several factors that may have contributed to these findings. The soil color and type between College Station and Corpus Christi are different which could cause plants to subsequently heat up at different rates. Concomitantly, the latitude and therefore accumulated solar radiation are different at these locations. Likewise, Corpus Christi experiences a coastal climate affected by the nearby ocean, whereas College Station is much further inland to be greatly affected by terrestrial heating and cooling effects from the ocean. Both temperatures in the morning and afternoon were different by year, which seems likely the result of the climate variation from year to year. There also were

Table 8. Mean square errors for drought tolerance traits across all locations and genotypes in 2015 and 2016.

Model Effects †	**d. f.**	**NDVI-AM**	**NDVI-PM**	**Temp-AM**	**Temp-PM**	**ACC**	**SC**
	-	-	-	C°	C°	µmol/m^2	mmol/m^{2}s
Genotype	13	.0198**	.0173**	1.367	2.880	6111.3	34523
Location	2	.3210**	.2469**	148.4**	10.11	738165.1**	1603095**
Rep [L]	9	.0224**	.3092**	4.179	9.031	1943.5	48375
Year	1	.6314**	.4160**	623.2**	1964**	15208.5	1295425**
G x E	26	.0044	.0041	.6231	2.254	2181.6	9103
G x Y	13	.0158**	.0168**	.4964	1.778	1648.6	28186
Growth Stage	2	1.684**	1.487**	40.20**	445.7**	434389.4**	2571637**
Error	1077	.0050	.0069	5.078	4.877	4869.8	37408
C.V. (%)	-	9.7	11.7	7.3	6.7	18.3	25.9

*, ** denotes significance at the .05, .01 level respectively.

† C.V., coefficient of variation; Rep, replication; L, location; G x E, genotype x location; G x Y, genotype x year; ACC, absolute chlorophyll content, SC, stomatal conductance; d. f., degrees of freedom

differences for leaf temperatures in the morning and afternoon for growth stages which stands to reason given the inherent changes in physiology as plants age.

Chlorophyll varied by location and growth stages. This too was likely the result of unique growing conditions at each location and changes in physiology as the plant matures. A similar explanation could be given for stomatal conductance that was affected by location, year, and growth stages

Years for each trait were analyzed separately due to the presence of significant year effects except for absolute chlorophyll content (Table 9). Average NDVI-AM and PM values were lower in 2015 than 2016. There was a wider dispersion of NDVI readings both in the morning and afternoon among genotypes in 2015 than in 2016. This can also be attributed to the reduced seed quality in 2015. With lower stands, NDVI will inherently be lower because there will be less biomass within the row. In 2015, Tamcot 73, 10X-64, DP 491, and 10X-78 were consistently higher than GP 130 for NDVI-AM and NDVI-PM.

There were no differences among genotypes for stomatal conductance in 2016 (Table 9). GP 79 had among the highest stomatal conductance which suggests it was actively sourcing and transpiring soil moisture. Conversely, GP 140 had among the lowest stomatal conductance which indicates that stomata were attempting to close in response to drought stress.

Leaf temperature, which can be an indirect measure of transpiration (Jackson et al., 1981; Lu et al., 1994; Singh and Kanemasu, 1983), was not different among genotypes in the morning or the afternoon in 2016 and not different in the morning in

2015 (Table 9). In 2015, the afternoon temperatures were different among genotypes. GP 561 had a leaf temperature of 32.2° C and GP 76 was 32.1° C which were among the warmest; whereas, Tamcot 73 had an afternoon temperature of 31.4° C which may indicate that it was more actively transpiring than some of the CRS lines. Interestingly, GP 561 had among the lowest absolute chlorophyll content at 356 µmol/m^2.

Table 9. NDVI, stomatal conductance, leaf temperature, and chlorophyll content of converted race stocks at College Station and Corpus Christi in 2015 and 2016.

Genotype ‡§	**NDVI-AM**		**NDVI-PM**		**Stomatal Conductance**	
	2015	**2016**	**2015**	**2016**	**2015**	**2016**
	-		-		----mmol/m^2s----	
Tamcot 73	.74a	.75ab	.72a	.72ab	834.0ab	732.3a
10X-64	.71abc	.75ab	.70abc	.73ab	765.3cde	708.1a
DP 491	.73ab	.75ab	.71ab	.72ab	794.7abcde	719.4a
10X-78	.71abc	.77a	.70abc	.74ab	776.9bcde	731.5a
GP 561	.69bcd	.75ab	.68abcd	.72ab	755.7cde	669.5a
GP 67	.71abc	.77a	.70abc	.75ab	753.2cde	686.5a
GP 79	.71abc	.77a	.70abc	.75a	856.4a	713.2a
GP 76	.69bcd	.75ab	.68abcd	.73ab	757.2cde	715.3a
GP 130	.66d	.73b	.65d	.71b	801.9abcd	666.3a
GP 116	.72abc	.73b	.71ab	.71b	793.3abcde	724.5a
GP 140	.66d	.76ab	.65cd	.73ab	725.8e	691.7a
GP 137	.70abcd	.75ab	.69abcd	.73ab	817.3abc	668.6a
GP 138	.68cd	.77a	.65cd	.74ab	736.2de	673.7a
GP 122	.68cd	.77a	.66bcd	.75a	813.0abcd	696.1a
Mean	.70	.76	.69	.73	785.4	700.3

† Mean values followed by different letters are significantly different at .05 level

‡ Entries are in order by lint yield, highest to lowest

§ Means are averaged across all locations within respective years

Table 9 continued

Genotype ‡§	Temp-AM		Temp-PM		Absolute Chlorophyll Content
	2015	2016	2015	2016	2015/2016
	----Cº----		----Cº----		µmol/m^2
Tamcot 73	29.9a	31.6a	31.4b	34.4a	384.7abc
10X-64	30.1a	31.6a	31.8ab	34.6a	393.7ab
DP 491	30.1a	31.6a	31.6ab	34.6a	377.6abc
10X-78	30.1a	31.4a	31.7ab	34.4a	398.2a
GP 561	30.4a	31.9a	32.2a	35.0a	356.3c
GP 67	30.3a	31.9a	32.0ab	35.3a	366.2bc
GP 79	30.1a	31.6a	31.6ab	34.2a	381.3abc
GP 76	30.3a	31.6a	32.1a	34.3a	379.8abc
GP 130	30.2a	31.7a	31.7ab	34.6a	368.9bc
GP 116	30.1a	31.9a	31.9ab	35.0a	384.1abc
GP 140	30.0a	31.7a	31.8ab	34.7a	380.9abc
GP 137	30.2a	31.9a	31.6ab	35.0a	369.9abc
GP 138	30.0a	31.5a	31.7ab	34.8a	378.4abc
GP 122	29.8a	31.4a	31.5ab	34.6a	390.2ab
Mean	30.1	31.7	31.8	34.6	379.5

† Mean values followed by different letters are significantly different at .05 level

‡ Entries are in order by lint yield, highest to lowest

§ Means are averaged across all locations within respective years

Differences among the locations were detected (Table 10) for every sensor related trait except NDVI taken in the morning and afternoon in 2016. In 2015 however, all three locations were different with Corpus Christi having the highest NDVI at .81 and College Station – dryland at .63. Locations were different for stomatal conductance in 2015 and 2016 with College Station - irrigated as the highest in 2015 and College

Station - dryland in 2016. Stomatal conductance was lowest at Corpus Christi in both years, which also had the lowest lint yield (Table 10).

Table 10. NDVI, stomatal conductance, leaf temperature, and chlorophyll content of converted race stocks across locations and years at College Station and Corpus Christi in 2015 and 2016.

Location ‡§#	NDVI-AM		NDVI-PM		Stomatal Conductance	
	2015	2016	2015	2016	2015	2016
	-		-		----mmol/m^2s----	
CS-D	.65c	.76a	.63c	.73a	781.1b	807.8a
CS-I	.68b	-	.68b	-	833.0a	-
CC	.81a	.75a	.81a	.73a	685.5c	575.6b
Mean	.70	.76	.69	.73	785.4	700.3

Location ‡§#	Temp-AM		Temp-PM		Absolute Chlorophyll Content
	2015	2016	2015	2016	2015/2016
	----Cº----		----Cº----		µmol/m^2
CS-D	30.6a	30.1b	32.7a	33.5b	423.6a
CS-I	30.4a	-	31.7b	-	313.1c
CC	29.2b	33.4a	30.3c	36.3a	372.8b
Mean	30.1	31.7	31.8	34.6	379.5

† Mean values followed by different letters are significantly different at .05 level

‡ CS-D, College Station – dryland; CS-I, College Station – irrigated; CC, Corpus Christi

§ Entries are in order by lint yield, highest to lowest

Means are averaged across all locations within respective years

Leaf temperature was the lowest at Corpus Christi both in the morning and afternoon in 2015 (Table 10). However, it was the location with the warmest leaf temperatures in the afternoon in 2016 at 36.3° C. All three locations were different in

terms of absolute chlorophyll content. The dryland test at College Station had the highest ACC at 423.6 µmol/m^2 and College Station irrigated at 313.6 µmol/m^2, which again may have been the result of data being collected in 2015 from plants in a thin stand.

In 2015, NDVI readings went higher as the plants aged, which suggests that vegetative cover was increasing without an appreciable difference in greenness in foliage (Table 11). In 2016 when drought stress was visible (e.g. wilting), NDVI values decreased from the flowering to the boll development stages. This drop in NDVI may have been a result of plants running low on nitrogen and/or the effects of drought stress upon chlorophyll content, which would in turn cause plants to be less green than healthier plants.

Across all genotypes, stomatal conductance in 2015 went from 623.0 mmol/m^2s at squaring to 809.1 mmol/m^2s at the flowering stage, which suggests that the more mature root system was accessing a greater amount of soil moisture therefore having a positive influence on transpiration (Table 11). In 2016, there was a large dip in stomatal conductance from the flowering to boll development stages as the values went from 869.7 mmol/m^2s down to 390.7 mmol/m^2s. Along with the drought stress of 2016, the additional boll load may have contributed to the precipitous decline in transpiration.

Leaf temperature in 2015 steadily declined as plants matured and developed (Table 11). This also may have been the result of root systems becoming more dynamic as the plants aged. However, in 2016 the reverse was observed as the leaf temperature rose as the plants aged with the afternoon temperature at the boll development stage being 38.2° C. The absolute chlorophyll content, which was averaged across years and

locations results, suggests that overall plants had more chlorophyll per leaf area once they started flowering. This may be attributed to robust root systems that could access more soil moisture, nitrogen and other nutrients.

Table 11. NDVI, stomatal conductance, leaf temperature, and chlorophyll content by growth stages of converted race stocks across locations and years at College Station and Corpus Christi, TX in 2015 and 2016.

Growth Stage §#	**NDVI-AM**		**NDVI-PM**		**Stomatal Conductance**	
	2015	**2016**	**2015**	**2016**	**2015**	**2016**
	-		-		----mmol/m^2s----	
Squaring	.51c	.70c	.49c	.69c	623.0b	848.2a
Flowering	.69b	.81a	.67b	.80a	809.1a	869.7a
Boll Development	.75a	.76b	.74a	.71b	796.7a	390.7b
Mean	.70	.76	.69	.73	785.4	700.3

Growth Stage §#	**Temp-AM**		**Temp-PM**		**Absolute Chlorophyll Content**
	2015	**2016**	**2015**	**2016**	**2015/2016**
	----Cº----		----Cº----		----µmol/m^2----
Squaring	32.7a	30.3b	34.6a	32.9b	346.4b
Flowering	30.4b	30.4b	31.8b	32.1c	386.4a
Boll Development	29.5c	34.3a	31.4c	38.2a	396.1a
Mean	30.1	31.7	31.8	34.6	379.5

† Mean values followed by different letters are significantly different at .05 level
§ Entries are in order by lint yield, highest to lowest
# Means are averaged across all locations within their respective years

Lint Yield

Lint yield usually is the most important economic trait for cotton production. Among the traits measured, NDVI was more frequently positively correlated to lint yield (Table 12). Both NDVI-AM and PM had significant and positive correlations within each location except for Corpus Christi in 2016.

In 2015 at College Station - irrigated and College Station - dryland, afternoon temperature showed a negative association with lint yield of -.40 and -.29 respectively, which indicates that entries with cooler leaf temperatures were also more productive in terms of lint yield. However, these relationships were not detectable in 2016.

College Station - dryland, 2015 also showed a moderate, yet still significant relationship (.15) between stomatal conductance and lint yield. With significant relationship between NDVI and temperature with lint yield, it speaks to the efficacy of these sensors for high throughput applications. These sensors are often used on UAV (unmanned aerial vehicle) to collect phenotypic information and these results demonstrate the application to drought tolerance as well.

Significant correlations for both NDVI-AM and NDVI-PM were observed during squaring (Table 12). The NDVI collected in the morning at the flowering stage were .54 and .43 respectively in 2015 and 2016; whereas the NDVI taken in the afternoon slightly dropped in its degree of correlation to lint yield. As NDVI is a function of biomass, plants that develop increased leaf area earlier in the growing season are more prepared for suppling the reproductive phases with resources, resulting in higher yields.

Table 12. Spearman's ρ correlations for lint yield by NDVI, leaf temperature, chlorophyll content, and stomatal conductance by year, locations, and growth stages of converted race stocks grown at College Station and Corpus Christi, TX, in 2015 and 2016.

Traits †	Location §					
	CS-I		CS-D		CC	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.48**	-	.50**	.18**	.52**	.02
NDVI-PM	.46**	-	.44**	.20**	.60**	-.04
Temp-AM (Cº)	-.18*	-	-.13	-.04	-.11	.01
Temp-PM (Cº)	-.40**	-	-.29**	.02	-.09	.01
ACC (µmol/m^2)	.06	-	.11	-.01	.18	-.03
SC (mmol/m^2s)	.09	-	.15*	.01	.10	.01

Traits †	Growth Stage					
	Squaring		Flowering		Boll Development	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.54**	.43**	.60**	-.41**	.32**	.00
NDVI-PM	.31**	.36**	.67**	-.38**	.35**	-.16*
Temp-AM (Cº)	-.14	-.02	-.20*	-.68**	-.12*	-.52**
Temp-PM (Cº)	-.37*	.06	-.28**	-.41**	-.29**	.08
ACC (µmol/m^2)	.06	.28**	.24	.64**	.00	.56**
SC (mmol/m^2s)	.12	.35**	.06	-.17*	.20**	.35**

Traits †	Year		
	2015	2016	2015/2016
NDVI-AM	.27**	.02	.18**
NDVI-PM	.29**	-.03	.16**
Temp-AM (Cº)	-.07	-.53**	-.24**
Temp-PM (Cº)	-.24**	-.36**	-.22**
ACC (µmol/m^2)	.02	.41**	.23**
SC (mmol/m^2s)	.13**	.31**	.21**

*, ** denotes significance at the .05, .01 level respectively

† ACC, absolute chlorophyll content; SC, stomatal conductance

§ CS-I, College Station – Irrigated; CS-D, College Station – Dryland; CC, Corpus Christi

Significant relationships during squaring demonstrate the importance of early season management for lint yield.

Absolute chlorophyll content and stomatal conductance show significant positive correlations to lint yield (.28 and .35) in 2016 at the flowering stage. Concomitantly with NDVI, chlorophyll content and stomatal conductance are indicators of photosynthetic capacity. With increased levels of chlorophyll and stomatal conductance, plants incorporate higher levels of carbon, increasing biomass. These findings may be a function of early-season plant health contributing to final lint yield.

The only significant correlation detected between leaf temperature and lint yield was in the afternoons of 2015 with a correlation -0.37. In the afternoon, plants typically are more stressed than during the morning due to increases in solar radiation and ambient temperatures. Since squaring occurred during June, when the most intense heat and drought stress had yet to occur, the lack of association between leaf temperature and lint yield is likely more a function of the lack of heat and drought stress than the inability of leaf temperature at this growth stage to predict lint yield.

While all three growth stages had correlations between traits and lint yield, correlations during flowering were usually of the greatest magnitude (Table 12). NDVI-AM and PM show strong positive correlations in 2015, yet show strong negative relationships in 2016. It is difficult to explain why this reversal in correlation was observed. Perhaps the change in association could be explained by the higher rate of a plant growth regulator in 2016, which inhibits vegetative growth in favor of reproductive

growth (Albers and Schnakenberg, 2016). This might have caused decreased NDVI levels with higher lint yields.

All correlations at the flowering stage between leaf temperature and lint yield were negative. In 2016, the morning leaf temperature had a correlation to lint yield of -.68 and the afternoon temperature had a correlation of -.41. During water stress, stomata will close and leaf temperature will rise due in part to a reduction in transpirative cooling. With closed stomata, squares, flowers and bolls can be dropped in an effort to preserve water.

In 2016, absolute chlorophyll content showed a strong positive (.64) relationship, which suggests that plant health in terms of leaf color promoted by chlorophyll content was important for lint yield. This may eventually be proven to be an important measureable trait by UAV platforms with RGB cameras.

During the plants' boll development and filling stage, positive correlations were observed for NDVI at both collection times in 2015 but not in 2016 (Table 12). Leaf temperature was mostly negatively correlated with lint yield, but the inverse relationship between leaf temperature and lint yield was not as pronounced as it was during the flowering stage. Interestingly, absolute chlorophyll content and stomatal conductance were correlated, .56 and .35 respectively, to lint yield in 2016. This may have been a reflection of the importance of plant health and function during the late season in the particular year.

In 2015, NDVI collected in the morning had a correlation of .27 with lint yield and NDVI from the afternoon had a correlation with lint yield of .29. NDVI data from

2016 was not correlated to lint yield. Leaf temperature from the morning in 2015 had a slight negative correlation to lint yield, but in 2016 the correlation of NDVI to lint yield at both collection times was stronger at -.53 in the morning and -.36 in the afternoon. Similarly, absolute chlorophyll content and stomatal conductance had positive correlations with lint yield, .41 and .31 respectively. When the data are combined from both years, all of the traits are significant.

Lint Percent

There were fewer correlations between lint percent and sensor-related traits in comparison to lint yield (Table 13). Because lint percent tends to be determined more so by genotype rather than in response to environment (Meredith and Bridge, 1971; Worley et al., 1974), it is not surprising that data collected from sensors that are reflective of environmental fluctuations were not effective at predicting cotton lint percent. In the College Station dryland test in 2015, correlation between lint percent and NDVI-AM was .20. At Corpus Christi in 2015, the NDVI at both collection times indicates a positive correlation with lint percent.

There were no correlations during squaring with lint percent possibly indicating that factors affecting lint percent occur during flowering and boll development (Table 13). Most of the correlations between lint percent and the sensor related data occurred during flowering, which suggests that plant health at floral initiation is critical to lint percent. In 2015 and 2016, NDVI-AM and PM at the flowering stage were positively

Table 13. Spearman's ρ correlations for lint percent by NDVI, leaf temperature, chlorophyll content, and stomatal conductance by year, locations, and growth stages of converted race stocks grown at College Station and Corpus Christi, TX, in 2015 and 2016

Traits †	Location §					
	CS-I		CS-D		CC	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.08	-	.20**	.07	.30**	.05
NDVI-PM	.05	-	.11	.08	.32**	-.02
Temp-AM (Cº)	-.04	-	-.02	-.02	-.04	.00
Temp-PM (Cº)	-.01	-	-.09	.03	-.10	.01
ACC (µmol/m^2)	.03	-	.10	.00	.05	-.02
SC (mmol/m^2s)	-.10	-	-.06	.03	-.03	-.01

Traits †	Growth Stage					
	Squaring		Flowering		Boll Development	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.17	-.04	.22**	.32**	-.15**	.03
NDVI-PM	.04	.00	.24**	.28**	-.18**	-.00
Temp-AM (Cº)	-.05	.05	-.06	.20*	.05	.22**
Temp-PM (Cº)	-.05	.06	-.12	.17*	.13*	-.01
ACC (µmol/m^2)	.16	-.06	-.13	-.28**	-.17**	-.25**
SC (mmol/m^2s)	-.16	-.10	-.01	.14	.06	-.20*

Traits †	Year		
	2015	2016	2015/2016
NDVI-AM	-.13**	.06	-.08*
NDVI-PM	-.12**	.05	-.06
Temp-AM (Cº)	.09*	.20**	.11**
Temp-PM (Cº)	.10*	.16**	.07*
ACC (µmol/m^2)	-.12*	-.16**	-.14**
SC (mmol/m^2s)	.01	-.11*	-.04

*, ** denotes significance at the .05, .01 level respectively

† ACC, absolute chlorophyll content; SC, stomatal conductance

§ CS-I, College Station – Irrigated; CS-D, College Station – Dryland; CC, Corpus Christi

correlated to lint percent. In 2016, leaf temperature was slightly correlated with lint percent at .2 in the morning and .17 in the afternoon.

During boll development in 2015, correlations between lint percent and NDVI-AM -.15, NDVI-PM was -.18 and absolute chlorophyll content was -.17 (Table 13). Lint percent was positively correlated with Temp-PM at .13. In 2016, Temp-AM and lint percent were correlated at .22, absolute chlorophyll content was at -.25, and stomatal conductance at -.20. Because lint percent is a ratio of lint to seed, unhealthy plants may have produced smaller seeds, which in turn may have increased lint percent.

Throughout 2015, significant negative relationships with NDVI-AM (-.13), NDVI-PM (-.12), and absolute chlorophyll content (-.12) were reported. Also in 2015, Temp-AM and PM showed positive associations, .09 and .10; similar to that in 2016, .20 and .16. Absolute chlorophyll content and stomatal conductance both had negative associations in 2016, (-.16 and -.11). Lastly, when the years are combined, NDVI-AM shows a significant negative relationship (-.08) Temp-AM and Temp-PM show positive relationships (.11 and .07), and absolute chlorophyll content reported a negative relationship (-.14).

Micronaire

Only negative correlations with micronaire existed among locations (Table 14). In 2015, NDVI-AM was significant in College Station - irrigated (-.16) and College Station - dryland (-.41), while NDVI-PM also was significant in College Station - dryland (-.36). Micronaire values are determined by the thickness of the secondary wall

within the cotton fiber and are readily influenced by environmental stress (Cotton Inc., 2016). An inverse relationship of fiber micronaire with NDVI would suggest that higher NDVI measurements, (e.g. more biomass/healthier) have lower micronaire values. Healthier plants likely partition resources more evenly between vegetative growth and fiber development as opposed to stressed plants that have less bolls to partition their resources.

Only NDVI was correlated with micronaire during squaring (Table 14). In 2015, both morning and afternoon NDVI measurements were negatively associated with micronaire while NDVI-PM in 2016 was positively correlated. This change in association could likely be caused by square abscission due to high insect pressure in 2016. As a result of stress, cotton will deposit photosynthate into the remaining bolls, elevating micronaire values. Therefore, in this instance, high NDVI values from the unaffected biomass will result in higher micronaire values primarily due to the reduction in squares.

During flowering of both years, NDVI measurements again negatively correlated with micronaire, indicating the overall health of these plants at this time (Table 14). A positive correlation of .33 with absolute chlorophyll content potentially shows a relationship between those plants with higher photosynthetic capacity and where the photosynthate is being deposited. The negative correlations with morning temperature (-.25) and stomatal conductance (-.28) are difficult to explain.

Table 14. Spearman's ρ correlations for micronaire by NDVI, leaf temperature, chlorophyll content, and stomatal conductance by year, locations, and growth stages of converted race stocks grown at College Station and Corpus Christi, TX, in 2015 and 2016

Traits †	Location §					
	CS-I		CS-D		CC	
	2015	2016	2015	2016	2015	2016
NDVI-AM	-.16*	-	-.41**	-.03	-.14	-.04
NDVI-PM	-.14	-	-.36**	-.01	-.09	.01
Temp-AM (Cº)	.01	-	-.01	-.04	.04	-.02
Temp-PM (Cº)	-.01	-	.02	.09	-.12	.00
ACC (µmol/m^2)	-.05	-	.24	.02	-.03	-.08
SC (mmol/m^2s)	.04	-	-.03	-.07	-.00	-.02

Traits †	Growth Stage					
	Squaring		Flowering		Boll Development	
	2015	2016	2015	2016	2015	2016
NDVI-AM	-.33*	.17	-.30**	-.29**	-.64**	-.01
NDVI-PM	-.35*	.23*	-.35**	-.31**	-.58**	-.04
Temp-AM (Cº)	.04	-.07	-.14	-.25*	.16*	-.22*
Temp-PM (Cº)	.07	.13	.11	-.01	.25**	-.01
ACC (µmol/m^2)	-.12	-.07	-.04	.33**	-.44**	.20
SC (mmol/m^2s)	-.25	.09	-.07	-.28**	.36**	.14

Traits †	Year		
	2015	2016	2015/2016
NDVI-AM	-.53**	-.01	-.42**
NDVI-PM	-.45**	-.02	-.35**
Temp-AM (Cº)	.20**	-.23**	-.02
Temp-PM (Cº)	.22**	-.11	-.04
ACC (µmol/m^2)	-.39**	.14*	-.18**
SC (mmol/m^2s)	.18**	.07	.14**

*, ** denotes significance at the .05, .01 level respectively

† ACC, absolute chlorophyll content; SC, stomatal conductance

§ CS-I, College Station – Irrigated; CS-D, College Station – Dryland; CC, Corpus Christi

While flowering and boll development are comparable, the relationships in boll development are of greater magnitude. All trait relationships with boll development were significant in 2015. NDVI correlations were negative in 2015 but with twice the magnitude of squaring or flowering, indicating that plant health during this period is crucial for low micronaire values. Positive temperature correlations in 2015 yet negative correlations during 2016 might be due to the presence of drought stress at during boll development in 2016.

In 2015, all traits measured were significant. NDVI-AM and PM showed -.53 and -.45 respectively; morning and afternoon temperature showed .20 and .22 respectively; and absolute chlorophyll content and stomatal conductance showed -.39 and .18 respectively. In 2016, only two relationships were significant. There was a negative relationship with Temp-AM (-.23) and a positive relationship with absolute chlorophyll content (.14). When all the data are combined, NDVI-AM and NDVI-PM have negative relationships (-.42 and -.35), absolute chlorophyll content is negative (-.18) and stomatal conductance is positive (.14).

Fiber Length

For fiber length (Table 15), relationships within location were only observed in 2015. In College Station - dryland, Temp-PM was negatively associated (-.26) with fiber length. This could be caused by high temperatures inhibiting fiber elongation. Corpus Christi, NDVI-AM and NDVI-AM show positive relationships, .35 and .40 respectively. Plants with higher biomass and health are able to produce longer fiber.

Table 15. Spearman's ρ correlations for fiber length by NDVI, leaf temperature, chlorophyll content, and stomatal conductance by year, locations, and growth stages of converted race stocks grown at College Station and Corpus Christi, TX, in 2015 and 2016

Traits †	Locations §					
	CS-I		CS-D		CC	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.08	-	.16	.09	.35**	.17
NDVI-PM	.05	-	.12	.04	.40**	.05
Temp-AM (Cº)	-.13	-	-.12	-.09	.01	-.01
Temp-PM (Cº)	-.02	-	-.26**	.00	-.02	.02
ACC (µmol/m^2)	.09	-	.15	.03	.13	-.05
SC (mmol/m^2s)	.05	-	.08	-.02	-.01	-.02

Traits †	Growth Stage					
	Squaring		Flowering		Boll Development	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.14	.33**	.17	-.07	-.10	.09
NDVI-PM	.20	.22*	.20	-.24*	-.08	-.03
Temp-AM (Cº)	.21	-.02	-.39**	-.42**	-.06	-.30**
Temp-PM (Cº)	-.24	-.01	-.11	-.23*	.00	.09
ACC (µmol/m^2)	.08	.11	.25	.37**	-.14	.31**
SC (mmol/m^2s)	-.03	.09	.15	-.13	.16*	.10

Traits †	Year		
	2015	2016	2015/2016
NDVI-AM	-.07	.10	.00
NDVI-PM	-.04	.00	-.02
Temp-AM (Cº)	-.03	-.31**	-.13**
Temp-PM (Cº)	-.04	-.20**	-.08
ACC (µmol/m^2)	-.13	.21**	.06
SC (mmol/m^2s)	.15*	.12*	.13**

*, ** denotes significance at the .05, .01 level respectively

† ACC, absolute chlorophyll content; SC, stomatal conductance

§ CS-I, College Station – Irrigated; CS-D, College Station – Dryland; CC, Corpus Christi

During squaring, NDVI-AM and PM show significant relationships in 2016 (Table 15) indicating that plant health in 2016 had a positive effect on fiber length. During flowering, negative relationships with morning and afternoon leaf temperature, -.42 and -.23 respectively, might be indicative of the sensitivity of fiber elongation to water deficits during this period (Dagdelen et al., 2008). Boll development shows a negative correlation (-.30) with Temp-AM and a positive correlation (.31) with absolute chlorophyll content in 2016.

In 2015, 2016, and in the combined years stomatal conductance shows a positive relationship with fiber length (Table 15). This consistency might demonstrate the importance of continued photosynthesis in determining final fiber length. In 2016 temperature was negatively correlated (-.31 and -.20) and absolute chlorophyll content was positively correlated (.21).

Strength

Two relationships among the locations showed significance for fiber strength (Table 16) but there was no consistency. In 2016 at College Station - dryland absolute chlorophyll content had a positive relationship of .22. NDVI-AM was also significant in 2015 with a positive relationship of .24.

Absolute chlorophyll content and stomatal conductance were the only two significant relationships during squaring in 2016. It logical for these traits to work in tandem as higher chlorophyll content means increased photosynthetic capacity. This translates to a higher amount of gas exchanged through the stomata. Morning and

Table 16. Spearman's ρ correlations for strength by NDVI, leaf temperature, chlorophyll content, and stomatal conductance by year, locations, and growth stages of converted race stocks grown at College Station and Corpus Christi, TX, in 2015 and 2016

Traits †	Locations §					
	CS-I		CS-D		CC	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.08		-.06	-.03	.24*	.14
NDVI-PM	.07		-.05	-.05	.21	.06
Temp-AM (Cº)	-.14		-.15	-.08	.04	-.01
Temp-PM(Cº)	-.06		-.18	.02	.14	-.01
ACC (µmol/m^2)	.04		.16	.22**	.09	-.05
SC (mmol/m^2s)	.04		.14	.03	-.08	-.02

Traits †	Growth Stage					
	Squaring		Flowering		Boll Development	
	2015	2016	2015	2016	2015	2016
NDVI-AM	.02	.17	.04	-.14	-.20	.07
NDVI-PM	-.03	.07	.07	-.22*	-.17*	-.06
Temp-AM (Cº)	.04	-.08	-.41**	-.41**	.03	-.29**
Temp-PM(Cº)	-.16	-.02	-.11	-.16	.07	.05
ACC (µmol/m^2)	.08	.31**	-.01	.52**	-.11	.38**
SC (mmol/m^2s)	.06	.24*	.10	-.19	.18*	.19

Traits †	Year		
	2015	2016	2015/2016
NDVI-AM	-.15**	.04	-.07
NDVI-PM	-.11*	-.03	-.08
Temp-AM (Cº)	.00	-.31**	-.12**
Temp-PM(Cº)	.03	-.20**	-.05
ACC (µmol/m^2)	-.10	.29**	.10*
SC (mmol/m^2s)	.14*	.16*	.15**

*, ** denotes significance at the .05, .01 level respectively

† ACC, absolute chlorophyll content; SC, stomatal conductance

§ CS-I, College Station – Irrigated; CS-D, College Station – Dryland; CC, Corpus Christi

afternoon temperature in 2015 and 2016 had negative relationships indicating that high temperatures likely have an antagonistic relationship with developing fiber. A .52 correlation in 2016 during flowering was reported with absolute chlorophyll content. For boll development, NDVI-PM reported a negative correlation of -.17 and a positive correlation of .18 for stomatal conductance in 2015. In 2016 however, a negative correlation with Temp-PM of -.29 and a positive correlation with absolute chlorophyll content of .38 was observed.

2015 showed negative correlations with both NDVI-AM and NDVI-PM with positive correlations with stomatal conductance. 2016 reported negative correlations for Temp-AM and Temp-PM with positive correlations for absolute chlorophyll content and stomatal conductance. In the combined analysis, a negative correlation with Temp-AM and positive correlation with absolute chlorophyll content and stomatal conductance were reported.

Conclusions

An important consideration when using correlations is to be able to sort between the mathematical coincidences and those that are indicative of relationships. Based on the literature, drought tolerant traits should behave in a certain manner when faced with water deficits. For example, NDVI should drop due to reduced biomass and photosynthetic rate (Gutierrez et al., 2012), leaf temperature should elevate due to reduced transpirative cooling (Hatfield et al., 1987), absolute chlorophyll content has not been studied enough to define a set response (Karademir et al., 2009), and stomatal conductance should decrease as the stomata close in response to water stress (Pask et al., 2012).

Using the framework purposed by Passioura (1996) and Condon et al, (2004), these sensor measured traits provide a mechanism for evaluating drought tolerance in terms of yield. NDVI provides an estimation of biomass (HI); stomatal conductance and chlorophyll content provide information about transpiration efficiency (WUE); and leaf temperature demonstrates overall water use as cooler plants are using more water (WU). When all four sensors are used together, plant breeders have the opportunity to better understand the interrelationships between the components of drought tolerance.

From field observations, the only time when water deficits were having an aesthetic effect was just after flowering and into boll development at both CS-D and CC locations in 2016. This is apparent when looking at the growth stage means separations in Table 11. There is a drop in NDVI and stomatal conductance and an increase in leaf surface temperature after flowering coinciding with the stress seen in the field. The

significant year effect in the model could have been caused by this differential drought stress. It is also difficult to directly compare College Station to Corpus Christi. As water deficits occurred at different times in each field, and weather patterns were mostly independent, the environmental effects will have different influences on drought tolerant traits.

The efficacy of whether is it better to collect NDVI and temperature measurements in the morning or evening is difficult to answer. NDVI-AM and PM followed nearly identical trends. Plants were more stressed in the afternoon than in the morning and therefore had lower NDVI readings because leaves were wilting. When looking at the mean separations, both NDVI-AM and PM detected comparative differences. For temperature it is a similar case. Temp-AM in 2015 and 2016 and Temp-PM in 2016 were unable to detect differences among genotypes. Collecting both morning and afternoon measurements from both of these sensors does create an opportunity to investigate changes in stress level based on daily environmental fluctuation.

While the converted race stocks are low-yielding compared to the cultivars and elite germplasm lines, a few of them perform comparable when these groups were screened for drought tolerance traits. GP 79, GP 122, and GP 137 were consistently near the top of the group, if not significantly greater in some cases for stomatal conductance, absolute chlorophyll content, leaf surface temperature, and NDVI. Since the CRS are so low yielding, yet comparable for the drought tolerance traits the possibility exists that either these lines are harboring linkage groups that negatively affect yield while

positively affecting drought tolerance traits, or the converted race stocks have accumulated alternate QTL's that could potentially be combined with the already high yielding varieties to improve their drought tolerance.

Significance in growth stages for each trait is centered on flowering. Lint yield showed its strongest correlation with NDVI, temperature and absolute chlorophyll content during flowering. Stomatal conductance, however, was correlated with lint yield during boll development which coincides with photosynthesis driving assimilate deposition in developing fibers. Lint percent also reaches its greatest magnitude in correlations during flowering but not to the extent of lint yield. As lint percent is often considered an important component of lint yield, lint percent's is likely a contributing factor to the greater significance of lint yield.

Micronaire is more convoluted than lint yield and the other fiber quality traits. Correlations with micronaire during boll development are more closely aligned with the assumptions of micronaire during drought stress. Inverse relationships with NDVI and absolute chlorophyll content coupled with positive correlations with temperature and stomatal conductance is indicative of plants that are using their bolls as a source sink. With drought stress, leaf temperatures are elevated, while a lower leaves and bolls are excised to reduce water use. The remaining leaves are still able to photosynthesize and deposit assimilates into the remaining bolls. Thus, water deficits lead to high micronaire and low NDVI from the reduction in biomass (Hake et al., 1990).

Fiber length and strength are more influenced by genetics than environment and follow similar trends among their relationships. Fiber length and strength reported

negative relationships with temperature and a positive relationship with absolute chlorophyll content during flowering and boll development.

In order to predict lint yield, lint percent, fiber length and strength, NDVI, temperature and absolute chlorophyll content measurements can be collected around the time when the crop is flowering. Stomatal conductance measurements should be collected during boll development for lint yield and micronaire.

Generation Mean Analysis

Within the four families, generation model effects were significant for the 10X-63/GP 137 population in regards to afternoon temperature, the 10X-64/GP 137 line for absolute chlorophyll content, and all lines, except for 07X26-3/GP 137, for lint yield (Table 17). NDVI in the morning and evening showed no significance for generation or replication during flowering. It is an important consideration of the GMA that there are significant differences between the generations within each family for an effective analysis.

Table 17. Mean square errors for drought tolerance traits and lint yield for all generation means analysis families at College Station, TX in 2016.

NDVI-AM

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	.0003	.0002	.0003	.0002
Replication	2	.0001	.0002	.0001	.0000
Error	10	.0001	.0003	.0001	.0001
C.V. (%)	-	1.2	1.9	1.3	1.1

NDVI-PM

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	.0001	.0002	.0001	.0002
Replication	2	.0001	.0003	.0002	.0002
Error	10	.0002	.0003	.0001	.0002
C.V. (%)	-	1.8	2.1	1.3	2.1

Temp-AM (Cº)

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	.30	.39	.07	.08
Replication	2	2.14**	.78	.54	.99
Error	10	.24	.45	.45	.29
C.V. (%)	-	1.6	2.2	2.1	1.7

Temp-PM (Cº)

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	.82**	.41	.69	.10
Replication	2	.43	1.40*	1.73	.65*
Error	10	.11	.30	.78	.15
C.V. (%)	-	1.0	1.7	2.8	1.2

*, ** denotes significance at the .05, .01 level respectively.

† C.V., coefficient of variation; d. f., degrees of freedom

Table 17 continued.

Absolute Chlorophyll Content (µmol/m^2)

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	1784.08	3687.94*	956.88	3640.35
Replication	2	857.09	2930.80	6553.67	2059.73
Error	10	2224.08	1057.69	3298.01	2283.44
C.V. (%)	-	8.5	6.0	10.8	8.8

Stomatal Conductance (mmol/m^{2}s)

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	6311.3	5629.17	6912.33	6524
Replication	2	132332.8**	98235.65**	86188.63**	67713.11**
Error	10	5123.4	2322.9	5051.6	3545.0
C.V. (%)	-	8.0	5.3	7.5	6.5

Lint Yield (kg/ha)

Model Effects †	d. f.	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
Generation	5	814333.8*	432920.6*	318310.3	563730.7**
Replication	2	263346.0	148858.4	205294.6	309482.4
Error	10	233178.0	98232.0	178954.0	95030.0
C.V.(%)	-	38.9	23.6	42.1	25.0

*, ** denotes significance at the .05, .01 level respectively.

† C.V. – coefficient of variation; d. f. degrees of freedom

Although regression analysis showed few differences among generations, mean separation shows that differences between generations do exist (Table 18). Generations in three out of four families showed differences for NDVI-AM, with only one family, 07X26-3/GP 137, being different for both NDVI AM and NDVI-PM. This might indicate that both parents in 10X-63/GP 137, 10X-64/GP 137, and 10X-78/GP 137 have the same alleles for response to afternoon stresses. For both Temp-AM and Temp-PM, only one family showed differences between generations, 10X-63/GP 137. The other three families have similar readings for both parents, indicating there may not be as much variation between the alleles in these families as there are in 10X-63/GP 137.

10X-64/GP 137 and 10X-78/GP 137 are the only two families that show differences between generations for absolute chlorophyll content. In both of these families, the F_1 mean is the lowest of the generations with similarities to one other generation, the F_2 in10X-63/GP 137 and the BCP_2 in 10X-78/ GP 137. Three out of four families show significant differences for stomatal conductance, 10X-63/GP 137, 10X-64/ GP137, and 10X-78/GP 137. In 10X-63/GP 137 and 10X-64/GP 137, the F_2 generation averaged greater than the parents, only being greater in 10X-63/GP 137. 10X-64/GP 137 and 10X-78/GP 137 have differences between the F_1 and BC generation. All four families show differences between generations for lint yield. 10X-64/GP 137 and 10X-78/GP 137 show differences between the parents. In terms of lint yield, the F_1 populations were not different from the parents. In the backcross to the higher yielding parent (BCP_1), yields were not different than the high yielding parent in 10X-63/GP 137, 10X-64/GP 137, and 10X-78/GP 137.

Table 18. NDVI, temperature, chlorophyll content, stomatal conductance and lint yield among generations in GMA families at College Station, TX in 2016.

NDVI-AM				
Generation ‡	**10X-63/ GP 137**	**10X-64/ GP 137**	**07X26-3/ GP 137**	**10X-78/ GP 137**
P1	.83a	.82a	.81ab	.83a
P2	.81b	.81a	.81b	.81b
F1	.83a	.80a	.82ab	.82ab
BCP1	.83a	.82a	.80b	.82a
BCP2	.83a	.82a	.83a	.83a
F2	.81ab	.82a	.81ab	.82ab

NDVI-PM				
Generation ‡	**10X-63/ GP 137**	**10X-64/ GP 137**	**07X26-3/ GP 137**	**10X-78/ GP 137**
P1	.81a	.81a	.79ab	.82a
P2	.80a	.80a	.80ab	.80a
F1	.80a	.79a	.81a	.80a
BCP1	.80a	.80a	.79ab	.80a
BCP2	.80a	.79a	.79b	.80a
F2	.80a	.80a	.80ab	.81a

Temp-AM (Cº)				
Generation ‡	**10X-63/ GP 137**	**10X-64/ GP 137**	**07X26-3/ GP 137**	**10X-78/ GP 137**
P1	30.7b	31.4a	31.0a	31.0a
P2	31.2ab	31.2a	31.2a	31.2a
F1	31.0ab	30.8a	30.8a	30.8a
BCP1	31.2ab	31.1a	31.0a	31.2a
BCP2	31.3ab	30.5a	30.9a	31.0a
F2	31.6a	30.6a	31.1a	31.0a

‡ P1 is the first parent in the cross, followed by P2

Table 18 continued

Temp-PM (C°)

Generation ‡	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
P1	32.9a	32.9a	31.5a	32.1a
P2	32.1b	32.1a	32.1a	32.1a
F1	31.8bc	32.3a	31.9a	32.0a
BCP1	31.3c	32.6a	32.8a	32.5a
BCP2	32.3ab	31.9a	31.7a	32.1a
F2	32.1b	32.0a	31.6a	32.0a

Absolute Chlorophyll Content (µmol/m^2)

Generation ‡	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
P1	570.1a	585.5a	556.6a	541.6ab
P2	541.3a	541.3a	541.3a	541.3ab
F1	548.4a	477.8b	513.5a	507.8b
BCP1	601.7a	542.9a	550.7a	549.8ab
BCP2	544.5a	551.7a	522.5a	514.2b
F2	538.9a	530.7ab	520.0a	605.8a

Stomatal Conductance (mmol/m^2s)

Generation ‡	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
P1	830.4b	940.8ab	896.5a	911.3ab
P2	898.3ab	898.3abc	898.3a	898.3ab
F1	879.8ab	868.4bc	946.0a	979.9a
BCP1	925.9ab	910.9abc	947.8a	850.4b
BCP2	896.1ab	844.0c	974.3a	880.8ab
F2	967.6a	959.5a	1023.5a	948.3ab

‡ P1 is the first parent in the cross, followed by P2

Table 18 continued

Lint Yield (kg/ha)				
Generation ‡	**10X-63/ GP 137**	**10X-64/ GP 137**	**07X26-3/ GP 137**	**10X-78/ GP 137**
P1	1293ab	1575ab	944ab	1502a
P2	781b	781c	781ab	781bc
F1	1726a	1500ab	1168ab	1737a
BCP1	1920a	1818a	1249ab	1488a
BCP2	1156ab	1261abc	1379a	1249ab
F2	578b	1033bc	505b	647c

‡ P1 is the first parent in the cross, followed by P2.

'ABCD' scaling tests were performed on families with significant differences between the generations for each trait (Table 19). Significance of the 'ABCD' test is determined at the .05 level. If any one of the four tests is significant, the three parameter model (only mean, additive, and dominance effects) is no longer appropriate and the six parameter model that includes epistatic effects (additive x additive, additive x dominance, and dominance x dominance) must be included.

Three families were tested for NDVI-AM and only one, 10X-63/GP 137 showed significance. One family was tested for significance in NDVI-PM, 07X26-3/GP 137, and it showed that the three parameter model is adequate. 10X-63/GP 137 was tested in both Temp-AM and Temp-PM and only the AM measurement requires the six parameter model. Two families were tested in absolute chlorophyll and only 10X-78/GP 137 demonstrated a need for the six parameter model. All families except 07X26-3/GP 137 were tested for stomatal conductance and they all show that the three parameter model is

Table 19. ABCD Scaling Tests for GMA families with differences among generations for NDVI, temperature, chlorophyll content, stomatal conductance, and lint yield measured at College Station, TX in 2016.

NDVI-AM

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	.01	-	-.03	.00
B	.02*	-	.03	.03
C	-.03	-	-.03	.01
D	-.03	-	-.01	-.01

NDVI-PM

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	-	-	-.02	-
B	-	-	-.03	-
C	-	-	-.02	-
D	-	-	.01	-

Temp-AM (Cº)

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	.71	-	-	-
B	.50*	-	-	-
C	2.52	-	-	-
D	.66	-	-	-

Temp-PM (Cº)

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	-2.11	-	-	-
B	.72	-	-	-
C	-.43	-	-	-
D	.48	-	-	-

*, ** denotes significance at the .05, .01 level, respectively.

Table 19 continued.

Absolute Chlorophyll Content (µmol/m^2)

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	-	22.57	-	50.15
B	-	84.19	-	-20.65
C	-	40.49	-	324.92*
D	-	-33.13	-	147.71*

Stomatal Conductance (mmol/m^{2}s)

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	141.62	12.67	-	-190.32
B	14.15	-78.66	-	-116.57
C	382.27	262.1	-	23.65
D	113.25	164.05	-	165.27

Lint Yield (kg/ha)

Test	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
A	820.56	560.93	386.90	-263.33
B	-195.86	240.56	809.74	-20.49
C	-3212.63	-1222.63	-1308.50	-3169.50
D	-1918.68	-1012.10	-1252.57	-1442.84

*, ** denotes significance at the .05, .01 level, respectively.

adequate. All four families were tested for lint yield and all families show that the three parameter model is adequate for partitioning the genetic effects.

Genetic variance effects are partitioned in Table 20. Of the three families that show differences among generations for NDVI-AM, only 10X-63/GP 137 showed a significant effect outside of the mean effect. 10X-63/GP 137 showed significant additive x dominance interaction effect, meaning that an additive effect at one locus is interacting with the dominance effect at another. 10X-63/GP 137 also showed an additive x dominance interaction for afternoon temperature. None of the families partitioned for Temp-PM, absolute chlorophyll content, and stomatal conductance had significant genetic effects. Two of the families, 10X-63/GP 137 and 10X-64/GP 137, had significant additive effects for lint yield, indicating there might be potential within these families for improving lint yield under drought stress.

Table 20. Partitioned genetic effects of GMA families for NDVI, temperature, chlorophyll content, and stomatal conductance, and lint yield at College Station, TX in 2016.

NDVI-AM

Effect †	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
m	.81**	-	.81**	.82**
a	.02	-	-.003	.02
d	.07	-	.04	.02
aa	.01	-	-	-
ad	.84**	-	-	-
dd	-.09	-	-	-

NDVI-PM

Effect †	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
m	-	-	.80**	-
a	-	-	-.003	-
d	-	-	-.01	-
aa	-	-	-	-
ad	-	-	-	-
dd	-	-	-	-

Temp-AM (Cº)

Effect †	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
m	31.5**	-	-	-
a	-.03	-	-	-
d	-1.27	-	-	-
aa	1.13	-	-	-
ad	31.6**	-	-	-
dd	.01	-	-	-

*, ** denotes significance at the .05, .01 level, respectively.

† m, mean; a, additive; d, dominance; aa, additive x additive; ad, additive x dominance; dd, dominance x dominance effects

Table 20 continued

Temp-PM (C°)

Effect †	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
m	32.1**	-	-	-
a	-.78	-	-	-
d	-1.61	-	-	-
aa	-	-	-	-
ad	-	-	-	-
dd	-	-	-	-

Absolute Chlorophyll Content (µmol/m^2)

Effect †	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
m	-	530.74**	-	605.85**
a	-	1.61	-	8.44
d	-	-19.29	-	-329.09
aa	-	-	-	96.83
ad	-	-	-	522.53
dd	-	-	-	265.92

Stomatal Conductance (mmol/m^2s)

Effect †	10X-63/ GP 137	10X-64/ GP 137	07X26-3/ GP 137	10X-78/ GP 137
m	967.62*	959.5*	-	948.25**
a	27.55	12.62	-	-47.9
d	-211.07	-379.28	-	-255.44
aa	-	-	-	-
ad	-	-	-	-
dd	-	-	-	-

*, ** denotes significance at the .05, .01 level, respectively.

† m, mean; a, additive; d, dominance; aa, additive x additive; ad, additive x dominance; dd, dominance x dominance effects

Table 20 continued

	Lint Yield (kg/ha)			
Effect †	**10X-63/ GP 137**	**10X-64/ GP 137**	**07X26-3/ GP 137**	**10X-78/ GP 137**
m	578.89	1033.41	687.87	646.91
a	1139.16**	1037.17**	468.15	706.97
d	4526.70	2346.00	2810.57	3481.12
aa	-	-	-	-
ad	-	-	-	-
dd	-	-	-	-

*, ** denotes significance at the .05, .01 level, respectively.

† m, mean; a, additive; d, dominance; aa, additive x additive; ad, additive x dominance; dd, dominance x dominance effects

Conclusions

Due to the nature of some of the traits analyzed and techniques used to gather the data in this study, the generation mean analysis was not effective at partitioning genetic effects among these families. NDVI measurements tend to lose variation among genotypes as plants develop, compressing the mean values of each generation. Absent discernable high and low parents and without adequate variation within each generation, the requirements of GMA are not met. A similar conclusion can be drawn for absolute chlorophyll content. There are no discernable differences between the parents and the lack of variation within the generations impedes the GMA. In regards to the analysis of variance tables, almost every family across all traits showed that ‘generation’ was not having a significant effect upon the model. In the case of some families for Temp-AM, Temp-PM, and stomatal conductance replication effect was the only significant effect. These three traits are readily sensitive to changes in the microenvironment that may have

occurred during data collection, and disrupted the ability of the model to detect differences among the generations.

Lint yield, however, is different from the other traits analyzed. Three out of the four families show differences among generations without a replication effect. In two families, 10X-63/GP 137 and 10X-64/GP 137, additive effects are significant, indicating sufficient genetic variation within these families to confer beneficial alleles for improving lint yield in drought stress conditions.

Factorial

For each of the drought tolerance traits and lint yield, an analysis of the model effects is detailed in Table 21. None of the model effects were significant for NDVI-AM but there was a significant interaction term for female x male indicating possible dominance variance in NDVI-PM. Temp-PM also shows the males were having a significant effect on the model. For absolute chlorophyll content, both male and female effects show significant contribution to the model. Lastly, in the case of stomatal conductance and lint yield, only replication significantly contributes to the model. As with temperature, significance among replications for stomatal conductance is more likely to be caused by changes in the environment during collection.

Table 21. Mean square errors of NDVI, temperature, chlorophyll content, stomatal conductance, and lint yield of factorial mating design in College Station, TX in 2016.

Trait †	Male	Female	Female x Male	Rep.	Error	C.V.
d. f.	3	1	3	2	14	-
N-A	.0001	.0003	.0005	.0002	.0002	1.9
N-P	.0003	.0000	.0015*	.0008	.0004	2.3
T-A	.13	.01	.10	2.0**	.23	1.5
T-P	.64*	.55	.30	1.0**	.14	1.1
ACC	4235.55*	19955.84**	447.96	1806.81	982.42	5.8
SC	7928.9	38444.0	4892.9	162810.0**	11998.6	12.0
LY	212818.0	5855.0	64623.0	1038055.0**	147652.0	27.8

*, ** denotes significance at the .05, .01 level, respectively.

† d. f., degrees of freedom; Rep, replication; C.V., coefficient of variation (%); N-A, NDVI-AM; N-P, NDVI-PM; T-A, Temp-AM (C^{o}) ; T-P, Temp-PM (C^{o}); ACC, absolute chlorophyll content (µmol/m^2); SC, stomatal conductance (mmol/m^{2}s), LY, lint yield (kg/ha)

Within this group, there were no differences among the progeny populations for lint yield, Temp-AM, and absolute chlorophyll content (Table 22). Only 10X-78/GP 76 is different from Tamcot 73/GP 122 and 10X-78/GP 140 for NDVI-AM. For NDVI-PM, 10X-78/GP 137, Tamcot 73/GP76, and Tamcot 73/GP 140 are higher than Tamcot 73/GP 137 and 10X-78/GP 140. Tamcot 73/GP 76 is lower than Tamcot 73/GP122 and 10X-78/GP 140 for Temp-PM. 10X-78/GP 137 and 10X-78/GP 76 are different than Tamcot 73/GP 122 and Tamcot 73/GP140 for stomatal conductance. Given that two crosses with 10X-78 were higher than two crosses with Tamcot 73, there could be interactions between QTL in these parents that are influencing performance of the progeny.

Utilizing the family structure within this group, variance component can be calculated (Table 23). Negative values were included but are assumed to be equal to zero. NDVI-AM and NDVI-PM both report dominance variation, yet more strongly for NDVI-PM. Temp-AM shows a positive additive variance component from the males in the group. Temp-PM also had this effect, but with additive components from the females and dominance effects. Absolute chlorophyll content reported positive additive variance effects from both male and female. Additive variance was detected from both male and female parents for stomatal conductance. Lint yield however, only showed additive variance components originating from the males.

Table 22. Fisher's LSD of factorial mating design of drought tolerance traits at College Station, TX in 2016.

Entry †	Lint Yield	NDVI-AM	NDVI-PM	Temp-AM	Temp-PM	ACC ‡	SC ‡
	kg/ha	-	-	Cº	Cº	µmol/m^2	mmol/m^2s
10X-78/GP 137	1737.0a	.82ab	.82a	31.0a	32.1ab	842.4a	583.1a
Tamcot 73/GP 137	1526.7a	.81ab	.78b	31.2a	32.2ab	966.1a	515.6bc
10X-78/GP 76	1515.8a	.84a	.81ab	30.8a	32.1ab	904.6a	590.0a
Tamcot 73/GP 122	1326.3a	.81b	.80ab	30.9a	32.3a	1010.0a	488.2c
Tamcot 73/GP 76	1307.8a	.82ab	.81a	30.8a	31.4b	963.2a	538.9abc
Tamcot 73/GP 140	1297.3a	.82ab	.81a	30.8a	32.2ab	868.6a	482.1c
10X-78/GP 122	1209.7a	.83ab	.80ab	30.6a	32.1ab	881.2a	524.8bc
10X-78/GP 140	1119.4a	.80b	.78b	31.1a	32.9a	859.5a	557.5ab
Mean	1379.9	.82	.80	30.9	32.1	911.9	535.0

† Mean values followed by different letters are significantly different at .05 level

‡ ACC, Absolute Chlorophyll Content; SC, Stomatal Conductance

Table 23. Variance partitioning for factorial mating design at College Station, TX in 2016.

Trait †	**V_m ‡**	**V_f**	**V_{mf}**	**$V_{A(Males)}$**	**$V_{A(Females)}$**	**V_D**
N-A	-.0001	-.0000	.0001	-.0002	-.0001	.0004
N-P	-.0002	-.0001	.0004	-.0008	-.0005	.0015
T-A	.0042	-.0073	-.0431	.0168	-.0291	-.1723
T-P	.0574	.0210	.0550	.2295	.0830	.2200
ACC	631.7	1626	-178.2	2525	6503	-712.6
SC	506.0	2796	-2369	2024	11183.7	-9474
LY	24699	-4897	-27676	98796	-19589	-110705

*, ** denotes significance at the .05, .01 level, respectively.

† N-A, NDVI-AM; N-P, NDVI-PM; T-A, Temp-AM (C^o) ; T-P, Temp-PM (C^o); ACC, Absolute Chlorophyll Content (µmol/m^2); SC, Stomatal Conductance (mmol/m^{2}s), LY, Lint Yield (kg/ha)

‡ V_m, variance from the males;V_f, variance from the female; V_{mf}, variance from the male x female interaction; MV_a, male additive variance; FV_a, female additive variance; V_D, dominance variation

Conclusions

The factorial mating design is useful for evaluating the genetic potential within and among separate groups. Tamcot 73 and 10X-78, a cultivar and an elite breeding line from the CIL, served as the high yielding female parents, while GP 76, GP 122, GP 137, and GP 140 were the male parents, representing different landraces within the larger CRS group that was evaluated. Two of the female parents, GP 122 and GP 137 were among the best performers for drought tolerance as determined by the HTP study. We are able to see how trait expression changes depending upon the generation, and determine sources of variation from this change. For Temp-AM, Temp-PM, absolute chlorophyll content and stomatal conductance; additive variance exists within the CRS group with potential to be incorporated into CIL's breeding program. There is variation still present within the CIL varieties for Temp-PM, absolute chlorophyll content, and stomatal conductance.

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